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ATOPIC DERMATITIS

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CONTENTS

PREFACE

First we would emphasize that this volume is not a complete record of all the lectures given at the Symposium.

Some authors have not submitted their papers for the present publication, and in other cases the lectures have been published elsewhere shortly before or since the Symposium. In most of these cases we reprinted the abstract and summarized the actual discussion.

The editorial work on the discussions after each paper was not easy. The need to keep expenses within limits forced us to shorten the discussions substantially. Questions about technical details, answered when reading the papers, were excluded and most of the questions were condensed. We sought to include and stress the essential points of the discussions, i.e. to give an abstract of the discussions, but in doing so, we may have quoted some inaccurately and misinterpreted others. We wish to apologize in advance for this and also to those whose substantial contributions to the discussions could not be given sufficient space.

Some participants in the discussions did not, as requested, give their names; they therefore appear only as "Q", i.e. question.

We thank all the contributors to this volume. We also express our gratitude to the chairmen who directed the sessions with great ability. Furthermore our thanks are due to Mrs N. Naalsund for her excellent secretarial assistance, and to the other members of the staff at the Department of Dermatology, Rikshospitalet, National Hospital, University of Oslo, for their valuable help and assistance. We also express our gratitude to the Editor of Acta Dermato-Venereologica, Professor N. Thyresson, for his kind help in arranging the printing of this Supplementum, as well as to the publishers, Almqvist & Wiksell, of Uppsala.

Oslo, February, 1980

Georg Rajka *Lasse R. Braathen*

OPENING

In the presence of 145 active participants from 16 countries, the first International Symposium on Atopic Dermatitis was opened with greetings from the Dean of the Medical Faculty, University of Oslo, Professor dr med Morten Harboe.

OPENING REMARKS

Arthur Rook

Professor Harboe, Professor Rajka, Ladies and Gentlemen,
I feel greatly honoured by the invitation to say a few words at the opening of this first International Symposium on Atopic Dermatitis. A symposium on this subject could not be more timely and Professor Rajka is to be congratulated on organising this meeting at a time when the international exchange of clinical and laboratory experience seems certain to give rise to fruitful discussion.

Since the concept of atopic dermatitis first began to take shape—and this was long before it received that name in 1933—it has generated an extraordinary number of hypotheses. These fall into two categories. The first category may be called legitimate. These are working hypotheses relating to one specific and defined aspect of atopic dermatitis, to be submitted to experimental investigation and thus to be confirmed or rejected. The second category may be called the hypotheses of self-justification. As physicians, we hate to appear to be empiricists and we need to think that we understand. We therefore formulate a hypothesis which combines what we believe to be scientifically established with a great deal of speculation in order to provide an apparently logical explanation for the treatments we employ and for the advice we give to our patients. I suppose that these self-justificatory hypotheses are in their own way legitimate too, provided of course that we never claim for them the scientific validity which they do not possess; provided also that we do not let our support for our favourite hypothetical concept lead us to ignore evidence which conflicts with it. Many dermatologists now accept that contact wealing is of great clinical importance in many patients with atopic dermatitis, and it is usually easy to detect, yet until Niels Hjorth drew our attention to it most of us had blindly overlooked it.

A surfeit of over-imaginative hypotheses is not the only handicap under which research on atopic dermatitis has laboured. Patients with atopic dermatitis may come under the care of paediatricians, allergists, dermatologists, or chest physicians, and specialists in each of these fields have

contributed largely to the literature. They often differ in their approach, in their findings and in their conclusions. It is easy to forget how different is the cross-section of atopic patients presenting to each of them, yet these differences account at least in part for divergent opinions which have sometimes been the subject of heated and even angry dispute.

That the development of atopic dermatitis depends to some extent on hereditary factors is generally accepted, but there is no agreement concerning the mode of inheritance. Most authorities believe it to be multifactorial. Unfortunately there is no agreement either as to the criteria for the diagnosis of the latent atopic state. There is evidence of marked geographical variation in the overall incidence of atopic dermatitis and there is evidence that some of this variation is genetic rather than environmental. There is evidence too that the relative incidence of the various physiopathological and immunological defects that combine to give rise to atopic dermatitis also differs between races and between large communities within a major racial group.

All these possible sources of variation must be borne in mind when we discuss the contributions we are about to hear on numerous aspects of the clinically important and scientifically fascinating problem that is atopic dermatitis.

WELCOME ADDRESS

Georg Rajka

Knowledge of the etiology and mechanism of atopic dermatitis is rapidly increasing and consequently this has often been reflected by the essential role of the disease as a topic on national and international dermatologic meetings. However, atopic dermatitis has not yet been discussed as an exclusive topic of a meeting with a tendency of evaluating and uniting the different aspects of the disease, in analogy to other dermatoses, e.g. psoriasis, although the world-wide distribution of atopic dermatitis would justify such a conference.

I therefore thought it worthwhile to organize this meeting as the first "International Symposium on Atopic Dermatitis", where experts from different countries will have a stimulating opportunity to contribute with significant data to the advancement of knowledge in this field. This will be of considerable interest, not only for dermatologists, allergists, immunologists, paediatricians and for other colleagues, but, most of all, of the utmost importance for the vast number of our patients suffering from this disease.

We will also do our best to take care of our visitors and show them Oslo and its lovely environs.

I hope that the occasion to meet old friends and to make new acquaintances will contribute to make this a pleasant stay for our guests and that we may continue the exchange of ideas on this field also in the future.

Welcome to Oslo.

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MORPHOLOGY OF ATOPIC ECZEMA

Nicholas A. Soter and Martin C. Mihm, Jr

Departments of Dermatology and Pathology, Harvard Medical School, the Divisions of Dermatology, Robert B. Brigham and Peter Bent Brigham Divisions of the Affiliated Hospitals Center, and the James Homer Wrigh Laboratories of the Department of Pathology and the Dermatology Service, Massachusetts General Hospital, Boston, Massachusetts, USA

Abstract. Atopic eczema, an inflammatory skin disorder characterized by acute vesicular lesions or chronic lichenified plaques, both accompanied by pruritus, occurs at any period of life in patients with personal or family histories of atopy. Previous histologic studies of atopic eczema using biopsy specimens stained with hematoxylin and eosin or with toluidine blue and ultrastructural studies of infantile eczema are now extended by studies using 1 μm thick sections stained with Giemsa's reagent. These biopsy specimens allow better definition of both normal skin structures and cells involved in the inflammatory response. This precision leads to clearer differentiation of diagnosis between the clinically similar atopic eczema and allergic contact dermatitis.

Key words: Atopic eczema; Mast cell; 1 μm thick sections; Allergic contact dermatitis

Atopic eczema (1) is an inflammatory skin disorder that occurs in patients with a personal and/or family history of atopy as manifested by asthma, allergic rhinitis, and rarely urticaria. Some patients have typical skin lesions without an atopic history. The disorder may begin during infancy, childhood, or adulthood. The acute lesions exhibit erythema, edema, and vesiculation that may lead to oozing. The chronic lesions are recognized as lichenified plaques with prominent skin markings. In older children and adults the lesions are typically localized to the flexural areas, especially the antecubital and popliteal fossae, and may be acute or chronic. Pruritus is the major symptom.

Histologic descriptions (2, 3) of atopic eczema have been based on studies of paraffin-embedded biopsy specimens stained with hematoxylin and eosin or toluidine blue and on ultrastructural studies of infantile eczema (4, 5). Use of 1 μm thick, glutaraldehyde-fixed, Epon-embedded sections (6–8) stained with Giemsa's reagent avoids the sampling problem inherent in electron microscopy and permits better definition of normal skin structures and cells of the inflammatory response than that which can be achieved in routinely processed specimens. This paper summarizes a description of the microscopic alterations which characterize atopic eczema (8) and compares these alterations with those reported in allergic contact dermatitis.

HISTOLOGY OF ATOPIC ECZEMA IN 1 μm SECTIONS

Skin biopsy specimens of the antecubital fossae were taken from individuals with atopic eczema whose ages ranged from 23 to 35 years. Specimens were obtained from acute vesicular lesions, from lichenified plaques, and from apparently normal skin, and processed as described (6–8).

Acute vesicular lesions (Figs. 1 and 2)

Epidermal hyperplasia with focal intercellular edema, vesiculation, and an epidermal infiltrate consisting predominantly of lymphocytes and macrophages were regularly observed. Compaction of erythrocytes in the superficial capillary venule without extravasation was noted. Marked perivenular and slight intervascular infiltrates were observed about the superficial venular plexi and consisted of lymphocytes, activated lymphocytes, and macrophages. Only occasional neutrophils, eosinophils, and basophils were noted; plasma cells were absent. Activated histiocytes were distributed throughout the superficial layers of the dermis and often contained melanin. Mast cells in acute vesicular areas occurred in normal numbers when compared with clinically uninvolved skin or skin from normal control individuals. Although endothelial cells of the superficial venular plexus were enlarged and contained large nuclei with clumped chromatin and prominent nucleoli, necrosis was not present. Vascular basement membrane alterations included edema, reduplication, and in some instances homogeneous thickening. Arterioles were normal.

Lichenified plaques (Figs. 3 and 4)

Hyperkeratosis, psoriasiform hyperplasia, and dyskeratosis of the epidermis were noted with focal areas of intercellular edema and infiltration by lymphocytes. Dermal edema was minimal, although compaction of the superficial capillary venule without red blood cell extravasation was noted. A moderate cellular infiltrate containing predominantly macrophages and lymphocytes was present in both perivenular and intervascular locations. The number of mast cells was significantly increased when compared to clinically un-

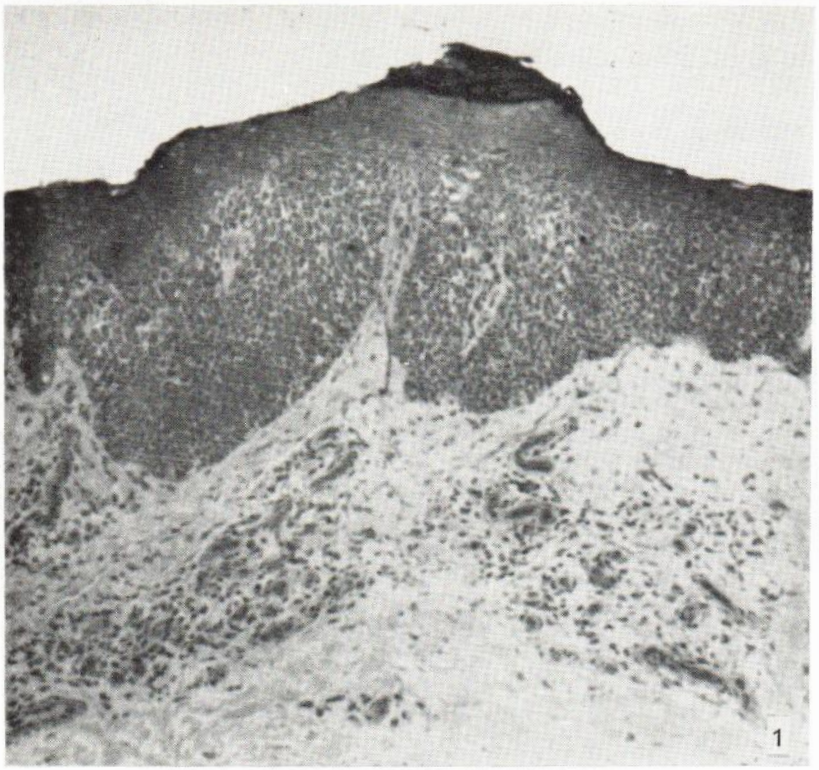
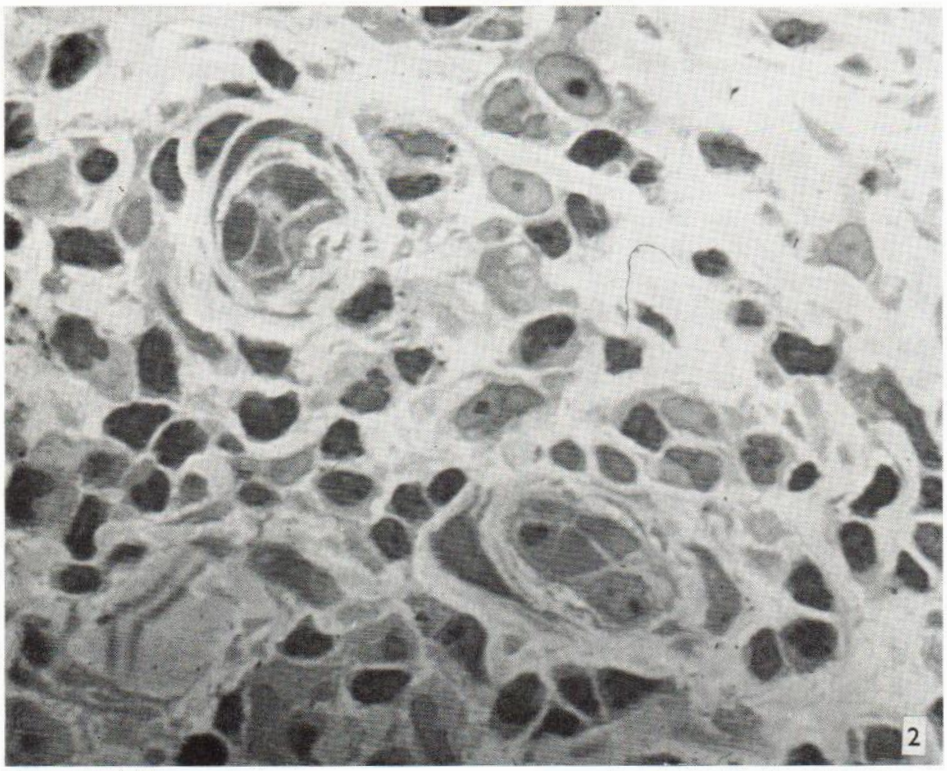
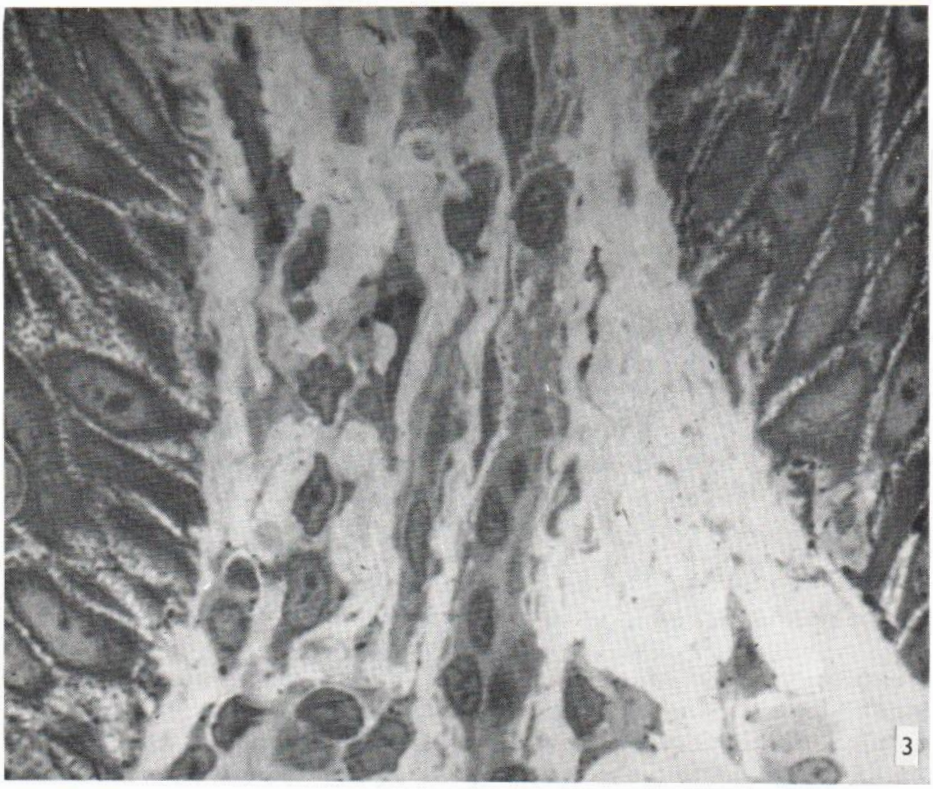


Fig. 1. Acute vesicular lesion with epidermal intercellular edema and a dermal perivenular infiltrate composed predominantly of lymphocytes. Giemsa, ×22.5.

Fig. 2. Acute vesicular lesion with lymphocytes disposed about venules which manifest hypertrophy of the endothelial cells and basement membrane reduplication. Giemsa, ×900.





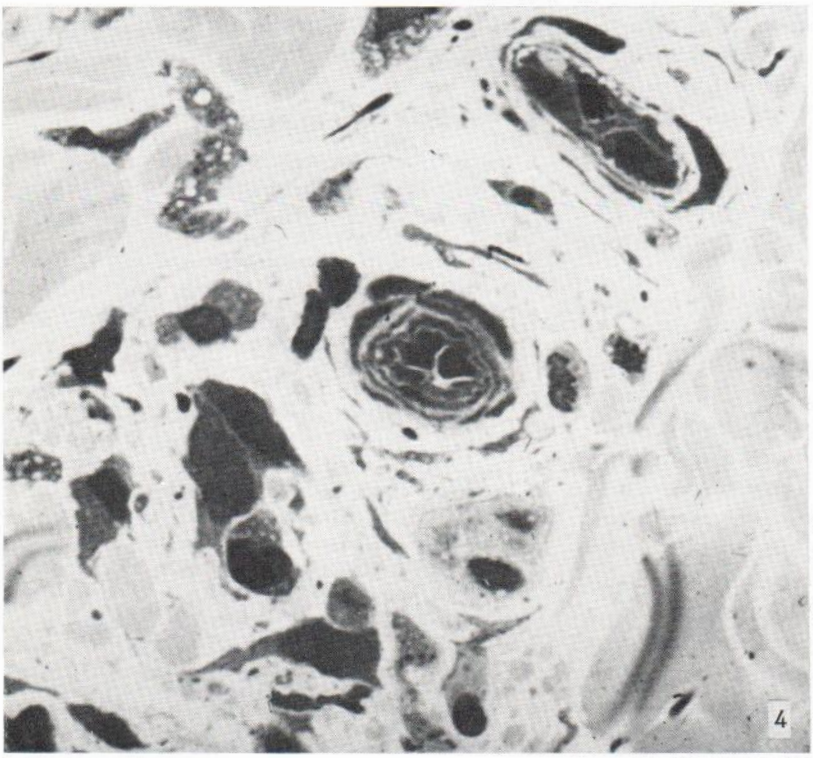


Fig. 3. Chronic lichenified plaque with focal epidermal intercellular edema and sclerosis of the superficial capillary venule. Note numerous macrophages. Giemsa, ×900.

Fig. 4. Chronic lichenified plaque with cutaneous nerve manifesting vacuoles, demyelination, and "ghost-like" remnants of nerve fibers. Note normal arteriole in center of field. Giemsa, ×900.

involved skin, acute lesions, or skin from normal individuals. Activated histiocytes were observed. Alterations of the superficial venular plexus and deeper venules included endothelial cell hypertrophy with enlarged nuclei and prominent nucleoli, basement membrane thickening, and enlarged pericytes. Cutaneous nerves at all levels of the dermis exhibited alterations including apparent demyelination and fibrosis. Occasional vacuolated areas appeared within their fibers.

Clinically normal skin

Traces of hyperkeratosis and epidermal hyperplasia, intercellular edema, and a slight dermal cellular infiltrate consisting primarily of lymphocytes were noted. Alterations occurred in most venules and included endothelial cell enlargement with focal luminal obliteration and prominent nucleoli, basement membrane thickening and/or reduplication, and enlarged pericytes. Sweat glands in all specimens appeared unaltered. Occasionally fibrosis and focal demyelination of cutaneous sensory nerves were noted.

DISCUSSION

Microscopic changes in the epidermis and dermis in atopic eczema vary with the nature of the clinical lesion. One μm thick biopsy sections from vesicular areas exhibited epidermal intercellular edema and a dermal inflammatory infiltrate of lymphocytes and activated lymphocytes disposed mainly about the superficial venular plexi. Venular alterations included endothelial cell hypertrophy with enlarged nuclei and basement membrane reduplication. In lichenified lesions there was epidermal hyperplasia with abnormalities of venules, increased numbers of lymphocytes and macrophages, and alterations in cutaneous nerves. In clinically normal skin abnormalities of the superficial venular plexi and venules similar to those of the lesional sites were observed.

The changes of atopic eczema in the 1 μm thick sections exhibit both similarities to and differences from those observed in allergic contact dermatitis (6). This method is valuable for differentiating between these two clinically similar types of eczematous dermatitis. In allergic contact dermatitis the changes in venules are noted only in relation to perivenular lymphocytic infiltrates, whereas in atopic eczema they occur both without and with a surrounding infiltrate. Allergic contact dermatitis manifests severe epidermal involvement and a dermal infiltrate which, in addition to lymphocytes and activated lymphocytes, exhibits more numerous basophils and eosinophils than are observed in atopic eczema. A striking difference between these reactions is the prominence of interstitial fibrin deposition in contact dermatitis (9) and its absence in atopic eczema. Moreover, the demyelination and fibrotic changes in cutaneous nerves found in lichenified lesions of atopic eczema have not been observed in contact dermatitis.

Previous descriptions of the venules in atopic eczema (3, 4) have been extended in this study by the recognition of endothelial cell hypertrophy, basement membrane reduplication, and homogeneous thickening. The alterations of venules in the absence of an inflammatory infiltrate in clinically normal skin may reflect previous involvement or, alternatively, an underlying vascular disorder.

The increase in absolute numbers of mast cells in lichenified plaques is compatible with the qualitative histologic observations previously made (3). It is also consistent with quantitative determinations of increased levels of tissue histamine in chronic lichenified plaques (10). Moreover, increased numbers of Langerhans' cells were noted in the epidermis of chronic lesions (11).

Cutaneous myelinated nerves from the lichenified lesional sites exhibited apparent demyelination and sclerosis without associated cellular infiltrates. Although the possibility of an ischemic mechanism must be considered, the derangement of neural structures may be related to the trauma to the skin incurred by repetitive scratching due to pruritus.

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DISCUSSION

Rørsman (Lund). Q: Can you differentiate between atopic dermatitis and atopic dermatitis with contact dermatitis in addition?

A: Such studies have not been performed.

Hanifin (Portland). Q: Is there any evidence of mast cell degranulation in acute atopic dermatitis, and have you made counts of mast cells in atopic dermatitis versus acute allergic contact dermatitis.

A: In acute vesicular areas the mast cells appeared variably hypogranulated, while those in lichenified plaques appeared full of granules.

Zachariae (Aarhus). Q: You found very few eosinophils. Would you think this is a marker that mast cell degranulation is not something very significant in atopic dermatitis in the acute stage?

A: No. Mast cells have at least 2 classes of eosinophil chemotactic factors. On the other hand, among lymphokines there are factors which are chemotactic not only for eosinophils but also for neutrophils and basophils, and we don't know what turns these on and off in the regulation.

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BACTERIOLOGY OF ATOPIC DERMATITIS

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Abstract. The aerobic bacterial flora of dermatitic skin, uninvolved skin, and the anterior nares of subjects with atopic eczema was investigated. A general comparison of the bacterial flora of subjects with atopic eczema, psoriasis and from a normal population was made. The incidence of *Staphylococcus aureus* in atopics was: 93%, 76% and 79% in the lesions, non-involved skin and the anterior nares respectively. For psoriatics the incidence was: 20% on the plaques, 13% on the uninvolved skin and 30% in the anterior nares.[1] The occurrence of *S. aureus* was very low on the skin of normal population (<10%). *S. aureus* was the predominant organism in the lesion of atopics and coagulase-negative staphylococci were the major bacteria in the psoriatic plaques and on the skin of the normal population. Fifty eight percent of the *S. aureus* strains isolated from atopics belonged to group 3 and 38% were nontypable. *S. aureus* strains belonging to phage groups 2 and 4 were not detected.

Key words: Staphylococcus aureus; Lipophilic diphtheroids

Bacterial flora of atopic dermatitis is strikingly different from the resident flora of normal skin (2, 5). The resident flora of normal skin consists mainly of coagulase negative staphylococci, micrococci and diphtheroids. Lipophilic diphtheroids are more common in the humid areas (axilla, groin and toe webs) than the dry skin. Gram negatives are noted only in moist humid anatomical sites.

Dermatitic skin provides favorable conditions for bacterial colonization and multiplication. The overgrowth of the normal flora and the colonization by certain pathogenic microorganisms not only present a threat to the patient, but also to those who come in contact with it. High occurrence of *S. aureus* in eczematous lesions have been reported (2, 5, 8). This investigation was designed to characterize quantitatively and qualitatively the aerobic microbial flora of atopic dermatitis.

MATERIALS AND METHODS

Thirty-nine subjects with an average age of 19 years were studied (range 4 to 52 years; median 16 years). Patients receiving antibiotics were not included. A majority (93%) of the lesions were of chronic, lichenified and non-exudative types. Subjects selected for this investigation were outpatients. The sites for sampling bacteria were the anterior nares, the inflamed skin area and the adjacent non-inflamed skin (normal skin). Forty patients with psoriasis and 20 normal subjects were included for comparison.

Skin samples were obtained by the detergent scrub technique (7). The nasal samples were collected with a calcium alginate swab. Serial dilutions were prepared and cultured on appropriate media. Organisms were identified according to their biochemical and growth characteristics (3). The Kirby-Bauer method was used for determining antibiotic sensitivity of *S. aureus* strains.

RESULTS

The incidence of bacteria on the atopics and the "normal population" is shown in Table I. The incidence of *S. aureus* was high in all the test sites of atopic patients. In the normal population this incidence was less than 10% on the skin. The occurrence of lipophilic diphtheroids was 2% on the uninvolved skin, 20% in the nose and none in the lesion; on the skin of normal population the occurrence of lipophilic diphtheroids was 47% at similar sites. The incidence of coagulase-negative staphylococci was not substantially different at the tested sites.

The mean density (39 subjects) of total aerobic flora was compared (Table II). *S. aureus* counts were higher both on the normal skin ($7.1 \times 10^3/cm^2$) and in the lesions (7.5×10^1 per cm^2). When present, the density of *S. aureus* on the skin in the normal population is extremely variable. *S. aureus* was the predominant organism in the lesion and constituted 91% of the total aerobic flora. On the uninvolved skin, coagulase-negative staphylococci were the predominant organisms (63% of the total flora). Lipophilic diphtheroids were not detected on the dermatitic skin and their counts were extremely low even on the normal skin ($6.7 \times 10/cm^2$).

Percent incidence of *S. aureus* was compared in

Table I. *Percent incidence of microorganism (39 atopics)*

	Anterior nares	Lesions	Normal skin	Normal population (skin) %
Staphylococcus aureus	79	93	76	< 10
Coagulase-negative staphylococci	77	79	82	80
Micrococci	2	13	25	40
Streptococci	2	0	2	0
Non-lipophilic diphtheroids	61	15	18	45
Lipophilic diphtheroids	20	0	2	47
Bacillus sp.	10	15	20	20
Gram-negative rods	2	5	5	20
Yeasts	0	0	2	< 1

atopics, in patients with psoriasis and in the normal population (Table III). The incidence of *S. aureus* was higher in all the three sites of atopics when compared with the sites of psoriatics and the normal individuals.

The antibiotic resistance of 140 strains of *S. aureus* was determined. Sixty three percent of *S. aureus* were resistant to two units and 58% to 10 units of penicillin. Resistance (20%) was also noted to 2 μg of tetracycline (14%) and 1 μg of oxacillin (8%).

Phage types of *S. aureus* are shown in Table IV. Thirty eight percent of the strains belonged to group 3, and 38% were non-typable. No phage group 2 or phage group 4 strains were detected. Different

Table II. *Average microbial counts in atopic eczema patients*

	Anterior nares	Lesions	Normal skin
Staphylococcus aureus	6.5×10^3	7.5×10^4	7.1×10^3
Coagulase negative staphylococci	1.4×10^4	7.1×10^3	1.5×10^4
Micrococci	0.8×10^3	1.6×10^2	9.5×10^2
Streptococci	3.7×10	< 10	0
Non-lipophilic diphtheroids	2.4×10^4	1×10^2	4.4×10^2
Lipophilic diphtheroids	5.4×10^3	0	6.7×10
Bacillus sp.	< 10	< 10	< 10
Gram-negative rods	< 10	< 10	< 10
Yeasts	0	0	10

Table III. *Percent incidence of* S. aureus

	Anterior nares	Lesion	Normal skin
Atopics (39 subjects)	79	93	76
Psoriasis (40 subjects)	30	20	13
Normal adult (30 subjects)	30	—	< 10

phage types were noted in the lesions, the non-inflamed skin and the anterior nares within the same subjects.

DISCUSSION

It was demonstrated that the incidence of *S. aureus* in the atopics was not only higher in the lesions (93%) but also in the anterior nares (79%) and non-involved skin (76%). The carriage of *S. aureus* on the skin (with the exception of perineum) of a normal population is less than 10% and in the nose ranges from 10 to 45%. Not only was the incidence of *S. aureus* higher in the lesions of atopics, but it constituted 91% of the total flora. This high density of *S. aureus* is not only hazardous to the patient but may also play an important role in the field of public health. Dispersion of *S. aureus* from inflamed skin has been noted in the hospital wards (4, 6).

The incidence (93%) and density ($7.5 \times 10^4/cm^2$) of *S. aureus* were much higher in atopic lesions than in psoriatic plaques (20% incidence and $3 \times 10^3/cm^2$ density (1). While *S. aureus* was the major organism in the atopic lesions, coagulase-negative staphylococci were the predominant bacteria in psoriatic plaques. The nasal carriage of *S. aureus* in psoriatics was comparable to that of the normal population.

Lipophilic diphtheroids are part of the normal skin flora. No lipophilic diphtheroids were detected in the lesions of atopics, and even on the un-

Table IV. *Phage types of* S. aureus

Group	Phage types ((%)
I	13
II	0
III	38
IV	0
Not typable	38
Not alloted (94/96)	12

inflamed skin their occurrence was minimal. The scarcity of lipophilic diphtheroids had also been noted in the psoriatic plaques (4% incidence) (1).

The finding that many strains of *S. aureus* (38%) colonizing the dermatitic skin belonged to group 3 was unexpected, since most staphylococci implicated in hospital epidemics in the 1950s and 1960s belonged to group 1. It is perhaps biologically fortunate for these patients that they have been colonized by group 3 and nontypable strains. Toxic epidermal necrolysis, bullous impetigo and recurrent furunculosis are most frequently caused by other than group 3 strains of *S. aureus*. The type 71 strain (group 2) has been associated with impetigo and toxic epidermal necrolysis. Whether colonization of *S. aureus* belonging to group 3 and non-typable groups was due to patient's lack of contact with the organism or an ecological preference over other strains of *S. aureus* is not known.

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DISCUSSION

Jones (Atlanta). Q: Is there any evidence that any components of the staphylococci which are on the skin are penetrating the epidermis?

A: We don't know because we did not do any sections.

Hanifin (Portland). Q. There is a peculiar reversal of the ratio between *Staphylococcus aureus* and lipophilic diphtheroids in atopic dermatitis. Could there be any relationship between this reversal?

A: We have seen the same in other conditions too. Lipophilic diphtheroids disappear first if topical antibacterials are put on the skin. These bacteria are extremely sensitive to harsh enviroments.

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ADRENOCEPTOR BINDING STUDIES WITH [^{3}H]DIHYDROALPRENOLOL AND [^{3}H]DIHYDROERGOCRYPTINE ON MEMBRANES OF LYMPHOCYTES FROM PATIENTS WITH ATOPIC DISEASE

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Abstract. Using [^{3}H]dihydroalprenolol (DHA) and [^{3}H]dihydroergocryptine (DHE), that is specific radioligands for the measurement of β- and α-adrenoceptors, respectively, binding studies were performed on membranes of lymphocytes from patients with atopic dermatitis, patients with non-atopic skin diseases, and normal individuals. A shift in the numbers of receptors from β to α was found in the lymphocytic membranes from patients with atopic dermatitis but not in the other 2 groups. That this shift cannot be due to possible variations in the proportion of lymphocyte subpopulations was demonstrated by the lack of difference in α- and β-receptor numbers between B and T lymphocytes, indicating that the adrenoceptor shift is intrinsic to the disease-state itself. Taken together with similar findings in lung tissue and lymphocytes from patients with reversible obstructive airways disease (asthma) the observations suggest that the constitutional basis of atopy may lie in an inherited or acquired adrenergic abnormality caused by a shift in available receptor numbers from β- to α-adrenoceptors.

Key words: Adrenoceptor; [^{3}H]dihydroalprenolol; [^{3}H]dihydroergocryptine; Binding; Lymphocytes; Atopy

In the late sixties, the beta adrenergic theory of the constitutional basis of atopy has reformulated the original concept of atopy. In its first (1), and more explicitly in its later expositions (2, 3), the theory, although primarily designed for human asthma, was stated to be also applicable to atopic dermatitis, since patients with the latter disease have increased serum IgE concentrations, a strong genetic predisposition to asthma, and exhibit qualitatively and quantitatively abnormal responses to pharmacological mediators or others stimuli as asthmatics do.

In the experimental analysis of the beta adrenergic theory, both chronologically and in research strategy, 4 phases can be distinguished. In the wake of the early presentations of the theory (1968–72), reduced asthmatic responsiveness measured by systemic parameters of β-adrenergic reactivity such as less rise in blood sugar, free fatty acids, lactate, pyruvate, pulse rate, urinary and plasma cyclic AMP levels and reduced eosinopenic response to β-adrenergic stimulation were found. In contrast, activities of adenylate cyclase, cyclic AMP phosphodiesterase, and cyclic AMP dependent protein kinase were found to be normal (4–6) in skin biopsies from the affected as well as normal-appearing areas of atopic dermatitis. On the other hand, in the subsequent years of 1972–75, in support of the earlier systemic findings in asthma, the same pattern of reduced β-reactivity was demonstrated in *in vitro* preparations of isolated cells derived from asthmatic individuals. In such studies, leukocytes and lymphocytes from asthmatic donors exhibited a reduced cyclic AMP response to beta adrenergic agents, but a completely intact response to other mediators suggesting that the biochemical defect is specifically beta adrenergic, and may in fact be at the level of the β-adrenoceptors. However, in the third phase of the experimental analysis of the theory (1975–77), it was shown that manifestations of β-adrenergic subsensitivity in asthma can be simulated, at least in part, in non-asthmatic normal individuals by pretreatment with adrenergic medication. These findings raised the question whether the adrenergic abnormalities seen in asthma may not be due to the disease itself, but rather to medication taken by asthmatic patients.

In the subsequent years (1977–79), however, beta adrenergic subsensitivity was found to be demonstrable also without concurrent medication, long after medication has been discontinued, and in lymphocytes derived from patients with atopic dermatitis (7) that is in an atopic condition in which adrenergic medication never has been involved. In addition, recent observations in our laboratory (8) provided conclusive evidence that β-adrenergic

Table I. *Binding of [³H]DHA and [³H]DHE to lymphocytic membranes from patients with atopic dermatitis*

Results of binding studies with [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE) to membranes of lymphocytes from normal controls, patients with nonatopic skin disease (NASD), and patients with atopic dermatitis (AD), at 11 nM DHA and 4 nM DHE concentrations using the methodology described by ref. 12

Group (N)	Ligand	Maximal binding fmol/mg protein	Ratio
Normal (70)	[³H]DHA	64±4	5.3
	[³H]DHE	12±5	
NASD (10)	[³H]DHA	58±8	5.3
	[³H]DHE	11±3	
AD (8)	[³H]DHA	36±6	0.5
	[³H]DHE	69±5	

subsensitivity must be regarded as a fundamental characteristic of atopy. In these studies, lung specimens obtained from patients with reversible airways disease (asthma) were shown a reduced capacity to synthesize cyclic AMP to β-adrenergic stimulation. That this abnormality must have lied at an early step of the biochemical sequence of β-adrenergic activation was evidenced by (a) the normal rates of phosphodiesterase-catalyzed cyclic AMP and cyclic GMP breakdowns in the same pulmonary specimens (9) and (b) by the marked shift in the numbers of adrenoceptors from β to α both in lung tissue as well as in membranes of lymphocytes obtained from the same patients with reversible airways obstruction (10).

Table II. *Binding of [³H]DHA and [³H]DHE to T and B lymphocytes from patients with atopic dermatitis*

Results of binding studies with [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE) to membranes of B and T lymphocytes from normal controls, patients with nonatopic skin disease (NASD), and patients with atopic dermatitis (AD), at 11 nM DHA and 4 nM DHE concentrations using the methodology described by ref. 12. Separation of T and B cell-enriched lymphocyte subpopulations was carried out by selective removal of cells forming rosettes with sheep red blood cells (T) or cells adhering to nylon wool columns (B) from whole peripheral lymphocyte populations. Studies were carried out on the non-rosetting (mostly B) or non-adhering (mostly T) cells in each case according to procedures described by refs. 13 and 14

Group (N)	Ligand	Maximal binding (fmol/mg protein) B cells	R	T cells	R
Normal (10)	[³H]DHA	62± 8	4.4	65±10	5.9
	[³H]DHE	14± 6		11± 4	
NASD (10)	[³H]DHA	61±10	5.1	57± 8	4.4
	[³H]DHE	12± 9		13± 4	
AD (8)	[³H]DHA	30± 2	0.4	32± 3	0.5
	[³H]DHE	69± 7		65± 5	

Using the same radioligands, that is [³H]dihydroalprenolol (DHA) and [³H]dihydroergocryptine (DHE), for the measurement of β- and α-receptors, respectively, the same shift from β- to α-receptors was found in membranes of lymphocytes from patients with atopic dermatitis, but not in lymphocytic membranes derived from patients with various non-atopic skin diseases (Table I). That this lymphocytic adrenoceptor shift cannot be due to possible variations in the proportion of lymphocyte subpopulations was demonstrated by the lack of difference in α- and β-receptor numbers between B and T lymphocytes, indicating that the adrenoceptor shift is intrinsic to the disease state itself (Table II). Furthermore, when saturation curves for the binding of DHA and DHE to adrenergically desensitized lymphocytic membranes from patients with asthma or atopic dermatitis were performed, the numbers of both adrenoceptors were quantitatively reduced, but the original ratios between the 2 types of adrenoceptors were preserved indicating that adrenergic desensitization is not the cause of the original adrenergic abnormality in lymphocytes of atopic individuals. Finally, pre-incubation of lymphocytes from atopic donors with hydrocortisone was found to restore the normal ratio between α- and β-receptor numbers (11).

These findings suggest that the constitutional basis of atopy both in asthma and atopic dermatitis may lie in an inherited or acquired (infection) adrenergic abnormality caused by a shift in available receptor numbers from β- to α-receptors possibly by mechanisms similar to those described for the phenomenon of adrenoceptor interconversion (3).

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DISCUSSION

Zachariae (Aarhus). Q: Have you done any longitudinal studies to investigate changes in the patients during longer or shorter periods of their lives?

A: It is possible that the basic status could change with time, especially when major hormonal changes take place in the body such as puberty, pregnancy and climacterium. Thyroid hormons, sexual hormons and insulin are able to shift the momentary balance between alpha/beta receptors, also triggering factors as e.g. viral infections may have a role here.

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USE OF *IN VITRO* EPIDERMAL CELL CULTURES TO STUDY GROWTH MECHANISMS IN HYPERPLASTIC SKIN DISORDERS

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Abstract. Both atopic dermatitis and psoriasis are proliferative skin disorders that are self-limited, show hereditary tendencies and are postulated to result from changes in the cyclic AMP-beta adrenergic system of the epidermis. Cyclic nucleotides, polyamines, arachidonic acid and its metabolites, and several drugs are associated with changes in the hyperproliferative epidermis. These biologically active compounds have been shown to affect the *in vitro* function of both neonatal and adult human primary culture systems. These *in vitro* model systems can be used to study the growth mechanisms of hyperplastic skin disorders.

Key words: Atopic dermatitis; Psoriasis; Cell model systems; Cyclic nucleotides; Prostaglandins; Polyamines

Two benign proliferative skin disorders used for biological studies are atopic dermatitis and psoriasis. As reviewed by Voorhees & Duell (16), a defect in beta receptor of atopic dermatitis has been postulated as an important aberration in this disease, i.e., the Szentivanyi beta-adrenergic theory of atopy, although investigations to date using biopsy epidermal tissues are still inconclusive (16). In psoriasis, a similar cyclic AMP-adrenergic receptor abnormality (5, 11, 17), along with changes in polyamine (13) and arachidonic acid metabolism (3, 4) have been demonstrated.

However, analyses of biopsy material can only suggest hypotheses that postulate the individual role, and the interplay of these factors and therapeutic drugs in the disease state. The investigation of these working hypotheses require isolated growing epidermal cells. Both a neonatal mouse and adult human epidermal keratinocyte system have been described and are presently being used in our laboratories to study epidermal cell function. We feel that these and similar technologies are reliable tools to study changes that may exist in the function of the psoriatic and atopic epidermis. In this report we will present some of the studies that have been done and are in progress using these *in vitro* systems.

METHODOLOGY

To prepare neonatal mouse primary cultures, the basal cells are trypsinized from 50 to 60 neonatal mouse skins. The basal cells are isolated from debris and any dermal fibroblasts using a discontinuous Ficoll gradient. Two to four $\times 10^6$ cells per cm^2 are plated in medium 199 + 13% fetal calf serum + antibiotics and grown at 31 to 32°C. The cultures proliferate, form multilayers (up to 10–11) and differentiate over a 4–5 week growth period. The exact details are presented in Marcelo et al. (12).

To establish adult human keratinocyte cultures, two 1×3 cm epidermal strips are removed from normal volunteers by use of a keratome. After trypsinization, approximately 1×10^6 cells in MEM + 10% calf serum + antibiotics are plated per 16 mm collagen coated well. After 24 h, the medium is changed to McCoy's 5A + 10% fetal calf serum + 4×10^{-4} M L-serine. The adult human cells grow, stratify and differentiate (at least, partially) at 37°C for up to 3 to 4 months. The details of this system are reported by Lui & Karasek (7).

DISCUSSION

The effect of elevated intracellular cyclic AMP levels on the neonatal mouse cultures has been extensively investigated (8). Briefly, we have shown that 1) both high, and moderate to low levels of cyclic AMP are associated with enhanced cell proliferation, 2) cyclic GMP (the other major biologically active cyclic nucleotide) is apparently not involved in the cyclic AMP stimulated growth, and 3) cyclic AMP enhanced keratinocyte growth is associated with increased specialization of the cultures.

Similar studies using the adult human epidermal cell cultures indicate that adult cells are also stimulated by increases in intracellular cyclic AMP levels although there is a marked difference in the dose and time response of the cultures (10). The most important aspects of these two investigations is the demonstration of an effect for cyclic AMP in both the neonatal and adult epidermis, thus suggesting a role for cyclic AMP in the pathology of the psoriatic and atopic epidermis.

As discussed, in psoriasis other mediators have been implicated in the disease process. Both the neonatal mouse and adult human systems are being used to study the association between arachidonic acid metabolites and epidermal cell growth. Hammarström et al. (4) report that the neonatal mouse keratinocyte in culture produces HETE (hydroxyeicosatetraenoic acid), prostaglandin (PG) E_2 and $PGF_{2\alpha}$, and that the epidermal hyperplastic agent TPA (tetradecanoyl phorbol acetate) stimulates cell production of PGE_2. This response, i.e., increased PGE_2 production, was prevented by the drugs triamcinolone acetonide and indomethacin. Sadowski (14), employing the same cultures, has similar findings and, in addition, reports that calcium ionophore A 23187 and retinoic acid also stimulated PGE_2 production. Sadowski & Marcelo (15) have reported PGE_2, $PGF_{2\alpha}$, PGD_2 and PGA_2 production by the adult human keratinocytes in culture which was inhibited by indomethacin. Both these cell systems possess arachidonic acid metabolic systems that may be suitable models for studies of keratinocyte growth regulation by these biologically active compounds.

The diamine and polyamine system, i.e., the levels of ornithine, putrescine, spermine and spermidine, and the enzymes involved in their interconversion: ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (SAMD) are augmented in the hyperproliferative psoriatic epidermis (13). By use of the neonatal mouse model system, the role of the polyamines in epidermal cell proliferation (6) and potential use of ODC inhibitors on epidermal cell proliferation is being studied.

In addition, drugs that can be therapeutic in hyperproliferative skin disorders (glucocorticoids; retinoids (2)) are being studied using the *in vitro* systems. Glucocorticoids have been demonstrated inhibit the proliferation of both normal and hyperplastic primary neonatal mouse epidermal cell cultures (9). Retinoid drugs may have both stimulatory and inhibitory responses (mouse and human) concomitant with changes in epidermal cell differentiation (1, 18).

CONCLUSION

Use of the primary neonatal mouse and adult human keratinocyte culture systems is contributing to our understanding of epidermal cell function, and of the changes in these functions induced by normal cell modulators and several drugs. The results of these studies can be applied to understanding the pathologies of the epidermal components of various cutaneous disorders, i.e., psoriasis and atopic dermatitis. However, the use of in vitro technology to grow the diseased epidermis itself will be necessary for a clear demonstration of the difference between the normal and diseased epidermis.

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DISCUSSION

Saurat (Paris). Q: Is atopic dermatitis an epidermal defect?

A: I don't know.

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BETA-ADRENERGIC BLOCKADE IN ATOPIC DERMATITIS. EVIDENCE OF AN ABNORMALITY OF T-LYMPHOCYTE BETA-RECEPTORS

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Key words: Beta-adrenergic blockade; E-rosettes; H-rosettes; Isoproterenol

β-adrenergic blockade in atopic dermatitis (AD): evidence of an abnormality of T-lymphocyte β-receptors.

During the last few years many data have been accumulated supporting the Szentivanyi theory of β-adrenergic blockade in atopic diseases. In particular it has been demonstrated that the responsiveness to isoproterenol of keratinocytes and leukocytes (evaluated as inhibition of DNA synthesis or as concentration of cAMP) is depressed in AD.

It is also known that intracellular levels of cAMP play a crucial role in the maturation and function of lymphocytes. Therefore it may hypothesized that T-cells of atopic patients undergo some disturbance in the stages of their maturation; this might explain the many recent reports of T-cell disfunction in AD.

We have presented evidence that T-cell differentiation in AD is likely to be disturbed: the percentage of lymphocytes forming rosettes with SRBC (E-rosettes) is lower, whereas the percentage of lymphocytes forming rosettes with autologous erythrocytes (H-rosettes) is higher then normal. It has been claimed that H-rosetting is a property of relatively immature T-cells (so-called T_1 cells), while further maturation to the more differentiated T_2 cells is accompanied by loss of this property. Isoproterenol inhibits the formation of H-rosettes, when added to lymphocytes of normal subjects, while it clearly fails to affect the percentage of H-rosetting lymphocytes from AD patients. These data suggest that the β-blockade could be responsible for the defect in the maturation of T cells. Moreover it has been demonstrated that isoproterenol inhibits the proliferation of lymphocytes induced by PHA, due to the increased concentration of cAMP.

We have shown that the percentage of inhibition induced by isoproterenol is lower in AD patients; the difference is statistically significant.

Propanolol antagonized the effect of isoproterenol, while PGE_1 produced the same effect in AD as in controls.

These data suggest that the β-receptor blockade affects the maturation, differentiation and function of T cells.

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CHARACTERIZATION OF β-ADRENORECEPTORS ON INTACT CIRCULATING LYMPHOCYTES FROM PATIENTS WITH ATOPIC DERMATITIS

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Abstract. [^{125}I]hydroxy benzyl pindolol (HYP) was used in a binding assay to compare the number and affinity of β-adrenergic receptors on circulating lymphocytes from 8 patients with severe atopic dermatitis (AD) and 8 age-matched controls. The number of receptors per lymphocyte (R_T) found in the atopic group (856 ± 132) was not statistically different from that of the controls (702 ± 107). By contrast, the dissociation constant (K_D) was significantly higher in patients with AD ($K_D = 20.8 \pm 3.3 \times 10^{-11}$ M) than in controls ($K_D = 12.6 \pm 3.45 \times 10^{-11}$ M). The results thus indicate that lymphocyte β-adrenergic receptors from patients with AD have a lower affinity for HYP than those of controls.

Key words: Atopic dermatitis; Peripheral blood lymphocytes; Beta-adrenoreceptors; [^{125}I]hydroxybenzyl pindolol

Although the pathophysiology of atopic dermatitis (AD) still remains uncertain, it is tempting to explain the combined cutaneous and immunological defects seen in this disease by a single cellular alteration. Several years ago, Szentivanyi proposed that the primary defect in atopy lies in an impaired cellular response to β-adrenergic agonists (26). Most of the data related to this hypothesis were obtained in pharmacological assays measuring either a metabolic, a physiological or a cellular response to β-adrenergic agonists. At the lymphocyte level, the presence of specific β-adrenergic receptors has been demonstrated in assays measuring either the synthesis of cAMP or the activity of the enzyme adenylate cyclase induced by various agonists in the presence or absence of antagonists (25). Methodological progress allowed the direct characterization of the β-adrenergic receptor on lymphocytic crude membrane preparations (27)—and even on intact living cells such as glioma cells (23), muscle cells (16) and human lymphocytes (20). In the present work we have used [^{125}I]hydroxybenzyl pindolol (HYP) to determine the number and the affinity of beta receptors present on peripheral blood lymphocytes (PBL) from patients with AD.

MATERIAL AND METHOD

Patients

Eight patients with atopic dermatitis (AD) (6 women, 2 men) were compared with 8 healthy subjects (1 woman, 7 men). The two groups were matched for age (mean: 28 years; range: 19–50). All the patients included in this study suffered from severe AD (more than 25% of body area involved with pruritus, excoriation and erythema or lichenification). The diagnosis of AD was based on the criteria proposed by Hanifin and Lobitz (10): pruritus, typical flexural lichenification and a chronic course.

The patients had elevated serum IgE levels (mean value: 765 UI/ml) as well as specific IgE antibodies to common allergens. All these subjects had a past history of asthma or allergic rhinitis. None had received systemic corticosteroids, beta adrenergic drugs or other anti-asthmatic treatment during the last 3 years.

The experiments were conducted in order to test simultaneously and under identical conditions equal numbers of patients and controls.

Lymphocyte preparations

The lymphocytes were isolated from heparinized blood by Ficoll-Hypaque centrifugation, macrophages and polymorphonuclears were removed by iron-powder treatment. The lymphocyte preparations contained less than 1% peroxidase-containing cells and almost no platelets. Viability of the cells was higher than 95%.

Method

Binding experiments were performed exactly as described previously (20). Briefly, $2\text{–}4 \times 10^6$ lymphoid cells were incubated for 60 min at 30°C in Hanks' Balanced Salt Solution (supplemented with 5×10^{-5} M phentolamine, 10 mM $MgCl_2$ and 50 mM Tris, pH 7.4) with varying amounts of [^{125}I.hydroxybenzyl pindolol ((±) ^{125}I-HYP). After 60 min, the mixture was diluted with 5 ml of 50 mM Tris HCl, pH 7.4, containing 10 mM $MgCl_2$ and was immediately filtered on a G F C Whatman fibreglass filter. The filter was washed with 25 ml of the same buffer at 37°C, and counted in a Packard gamma counter (efficiency 65%). The radioactivity bound in the presence of 10^{-7} M cold HYP or 10^{-7} M (−) propranolol was considered as the "non-specific binding".

The specific binding to lymphocytes was defined as the difference between the total and the non-specific binding. The number of receptors and the dissociation constant (K_D)

Table I. *Receptors on peripheral blood lymphocytes in patients with atopic dermatitis and in controls*

R_T = number of β-receptors per circulating lymphocyte in normal subjects and in patients with atopic dermatitis; K_D = the dissociation constant for [^{125}I]-HYP binding. Mean ± S.E.M.

	Patients	Controls
N	8	8
R_T	856 ± 132	702 ± 107
K_D	$20.8 \pm 3.31 \times 10^{-11}$**	$12.6 \pm 3.45 \times 10^{-11}$

** $t = 6.23$, $p < 0.01$.

were calculated by using a Scatchard-plot calculated from the saturation curve. All measurements were done at equilibrium, which is reached after 30 min incubation and is stable for at least 90 min (20). Previous studies (20) have shown that under these experimental conditions, the lymphocyte binding sites displayed the saturability and stereospecificity expected of true beta adrenergic receptors. The displacements observed with three agonists (isoproterenol, epinephrine, norepinephrine) further showed an order of potency compatible with a beta-2-type of receptor.

RESULTS

As shown in Table I, peripheral blood lymphocytes from normal subjects displayed 702 ± 107 receptors per cell with a K_D of $12.6 \pm 3.4 \times 10^{-11}$ M. In the atopic group, the number of receptors per lymphocyte was comparable (856 ± 132) whereas the K_D was significantly higher ($20.8 \pm 3.3 \times 10^{-11}$ M; $p < 0.01$).

DISCUSSION

Logsdon et al. (12), Parker & Smith (18) and Alston (1) reported that lymphocytes from asthmatic patients had a selective, depressed response to beta adrenergic agonists. The interpretation of their findings is difficult, because most of the patients were under sympathicomimetic treatment, which is known to depress the leukocyte response to adrenergic agents (5, 16). Parker & Eisen (17), working on patients with AD who were not receiving bronchodilator therapy, have shown that maximum concentration of isoproterenol increased cAMP concentrations five-fold in leukocytes of normal subjects, but only two-fold in leukocytes from AD patients. Similar findings have been made by Reed (21). In the present study, we compared the number and the affinity of beta adrenergic receptors on intact circulating lymphocytes from patients with AD and from healthy controls of same age.

We found that PBL from healthy young adults display 700 receptors per cell; these results are very close to those of Sano (22) and to our earlier findings in intact tonsil lymphocytes (20). The data clearly show that the β-receptors on lymphocytes from patients with severe AD are not reduced in number but have a significantly lower affinity for [^{125}I]HYP. A direct conclusion from these results would be that the aforementioned findings of Parker and Eisen (17) and Reed (21) cannot be explained by a reduction in the number of leukocyte adrenoreceptors. A first objection to this interpretation could be that the patient's lymphocytes contain abnormal proportions of B and T cells (8, 13, 2, 14, 3, 4, 11). This argument would be particularly relevant in the light of our recent findings that T lymphocytes have many lower beta receptors than B lymphocytes (20). However, we (6) and others (9) could not find any reduction in the T cell content of PBL from patients with AD. A second possibility to be considered is the influence of topical corticosteroid treatment on the beta receptors. Indeed, all the patients included in this study had been treated for several days with two applications per day of corticosteroid ointments on more than 25% of the body surface. The absorption of steroid hormone is certainly not negligible under such clinical conditions. The possibility that this treatment might interfere with the number of beta receptors is strongly suggested by recent in vitro and in vivo findings that hydrocortisone induces the synthesis of new beta receptors (7, 15) without altering the K_D.

Thus it is still possible that untreated atopic patients have a reduced number of lymphocyte beta receptors and that this anomaly is corrected by local corticosteroid treatment.

Our findings of a significant difference in the K_D for [^{125}I]HYP between atopics and controls cannot be explained by either an eventual decrease in T cell number or the use of topical steroids. Indeed peripheral T and B lymphocytes do not differ in their K_D for [^{125}I]HYP (20) and hydrocortisone has no effect on this parameter (7, 15).

The present results showing a normal number but a reduced affinity of β-receptors in lymphocytes from atopic patients should be compared with those of Singh et al. (24). These authors, working on the maturation of mice thymocytes, observed a progressive reduction during the maturation process of

the thymocyte cAMP synthesis in response to isoproterenol. This could not be accounted for by a modification in the number of receptors but was paralleled by a progressive reduction in the affinity for [^{3}H]alprenolol, used to label the receptor sites.

We suggest on the basis of our findings in this study that there is a qualitative anomaly of the β-receptors in the lymphocytes from atopic patients, although we cannot exclude the possibility of a quantitative defect. Further experiments are necessary before a definite conclusion can be reached. For example, the K_D of various agonists should be compared in displacement experiments on cells from atopics and controls. Another basic question to be answered is the conceivable effect of the inflammatory mediators released in the patients on the function and expression of β-receptors.

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DISCUSSION

Dobson (Buffalo). Q: Are the changes in lymphocytes secondary to the disease or do they represent the primary change?

A: The changes in atopic dermatitis are very similar to the changes that have been found in the lymphocytes in psoriasis, and where there seems to be a clear relationship between the severity of the disease and the impairment of the lymphocytes functions. The suggestion has been made that

the changes in lymphocytes are therefore secondary to the inflammatory disease, rather than the primary cause. We studied patients in a very mild phase and an active phase and found no differences between the two groups. We have followed up the patients for one year and examined them in different phases of activity of their dermatitis and there are no differences in the data with regard to isoproterenol response and PHA responsiveness. Family studies should be performed to investigate if this defect is genetic, or due to inflammation.

Strannegård (Gothenburg): We have some data regarding the primary or secondary nature of the lymphocyte aberrations seen in atopics. A prospective study on newborn infants has been performed and a significantly lower number of T-lymphocytes was found in one-month-old children with atopic parents as compared with newborns with nonatopic parents. This argues strongly for the primary nature of the lymphocyte deficiency in atopy.

Voorhees (Ann Arbor): The basic question is not whether the defect is primary or secondary, but whether it is important or not. Most common diseases are an interaction of multiple genes with their environments and a way of approaching this on the molecular level is really not available in modern science at the moment.

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SMALL VESSEL REACTIVITY IN ATOPIC DERMATITIS

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Abstract. The vascular reaction following local application of thurfyl nicotinate and i.d. injection of 10^{-2} mg metacholine were studied. Temporal variations in response and multiple patterns were found. Erythematous skin (increased amount of blood in the subpapillary venous plexus) always corresponded to arteriolar dilation, but in blanched skin an arteriolar fluctuation between dilatation and constriction could easily be followed. A stronger tendency to vasoconstriction was recorded in involved vis-à-vis uninvolved skin.

Key words: Vascular reactivity; Reflectometry; Photoelectric plethysmography

Several authors have investigated and described altered vascular responses to acetylcholine and nicotinic acid esters, especially thurfyl nicotinate in atopic dermatitis (1, 2, 3, 4, 5). In almost all instances the reactions were evaluated visually and the delayed blanch response was generally interpreted as a tendency to vasoconstriction. It was not until 1969 when Ramsay (6) performed objective measurements by means of plethysmography that this theory was challenged.

In the present study we have performed continuous reflectometric measurements of the state of the subpapillar venous plexus, while the arteriolar response was evaluated plethysmographically.

MATERIAL AND METHODS

Twenty patients of both sexes aged 11 to 30 years and suffering from atopic dermatitis, were studied. Only those who had not been treated with fluorinated steroid creams were admitted.

Reflectometry. Changes in skin colour on the volar aspect of the forearms were registered by photoelectric reflection meter (Photowolt 670). Preliminary, comparative measurements by this method have proven a high degree of reproducibility, superior to visual evaluation. The skin colour as measured by reflectometry depends on the amount and distribution of pigment, the colour of the surface layers of the epidermis, and filling of the subpapillary venous plexus. Only the latter is of significance in comparative short measurements. An increased reflection on the photometer is produced by a blanching which is a sign of decreased blood flow in the subpapillary venous plexus. The red tinge is caused by a dilatation of these vessels and lower values are registrated reflectometrically.

Photoelectric plethysmography. This is actually a pulse meter which measures the pulsating arteriolar blood flow (7). An increased pulse amplitude corresponds to an ateriolar dilatation in the dermis, and vice versa. Fig. 1 represents a schematic drawing of the two instruments. Reflectometric and plethysmographic registrations were performed on uninvolved and involved skin of the forearms. Metacholine (acetyl-beta-methylcholine) 10^{-2} mg in 0.1 ml of physiologic saline, was injected intradermally in one arm. Thurfyl nicotinate (Trafuril® ointment 5%) was gently rubbed into a measured area of the contralateral arm for 30 sec and wiped off with alcohol on cotton. Registrations with both methods were repeatedly performed for 30–60 min.

RESULTS

The findings are illustrated in Table I.

Uninvolved skin. With reflectometry, varying data were recorded: 8 out of 14 patients showed either increased (5 patients) or almost unchanged (3 patients) values following thurfyl nicotinate application. These patients with delayed blanch or visually unchanged (pale) skin, represent the paradoxical reaction characteristic of atopic dermatitis. Increased pulsation, i.e. arteriolar vasodilatation, was observed in 13 of the 14 patients. In one patient the pulse amplitude varied, but with a tendency to increased values after 15 minutes.

Involved skin. A higher reflection percentage occurred in 4 out of 6 patients following application of thurfyl nicotinate and in 3 patients following methacholine injections. This corresponded to a visually observed blanching. In the other cases varying degrees of erythema and decreased amounts of reflection were noted. In 3 out of 6 patients a clear decrease in pulsations (arteriolar vasoconstriction) occurred following thurfyl nicotinate and methacholine. In the others the pulse amplitudes were either unchanged or alternatingly decreased or increased.

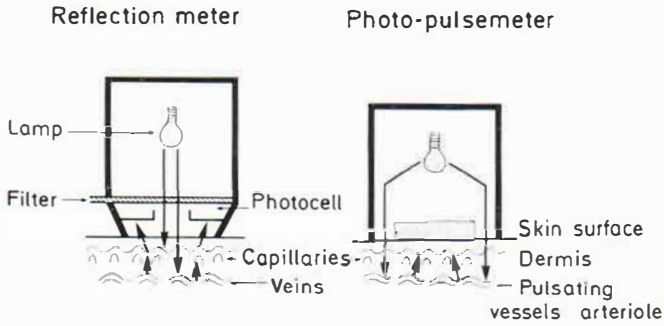

Fig. 1

DISCUSSION

The usual cutaneous reactions in normal persons to thurfyl nicotinate and metacholine are dilatation of the pulsating arterioles in the dermis and of all the other vessels governing the colour of the skin, in particular the subpapillary venous plexus. In the present study some patients reacted in both involved and uninvolved skin of the forearms, as do normals. But particularly in involved skin a variability of vascular changes was observed by both methods. This lability of vascular tonus or dynamic course of the reaction might be dependent upon the skin area studied (regional variations in vascular pattern) and the degree of inflammation in this area.

Although the reactions to thurfyl nicotinate and methacholine differed in a few patients, the similarity in vascular pattern points to some common or related vasoactive substances, although different mechanisms have been suggested (2).

Our results differ somewhat from those obtained by Ramsay (6) (see Table I) and indicate a more fluctuating vascular reaction, in particular of the deeper vessels. It seems important to distinguish between the reactions in these vessels and the more superficial vascular patterns, as they may react quite differently. This phenomenon is quite typical in urticarial wheals where the oedema can compress the superficial vessels, giving rise to blue, red, or white areas. But despite these colour changes there is considerable vasodilatation of the deeper dermal vessels (personal observations). This vasodilation in urticaria is far more prominent than in atopic dermatitis.

Altered vascular reactions to methacholine and nicotinic acid esters have recently also been described in dermatitic changes of allergic contact type (8). This report suggests that the abnormal vascular reactions in atopic dermatitis are secondary phenomena. Further studies are needed to elucidate this problem.

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Table I. *Results of present study and of Ramsay (6) on paradox vascular reaction in atopic dermatitis*

Uninvolved skin			
Trafuril (1–15 min)	8/14:	blanching/increased pulsation	6/14 (as normals): redness/increased pulsation
Involved skin			
Trafuril (1–15 min)	3/6:	blanching/decreased pulsation	2/6 (as normals): redness/increased pulsation[a]
	1/6:	blanching/increased pulsation	
Methacholine (5–25 min)	1/6:	blanching/unchanged pulsation[a]	2/6 (as normals): redness/increased pulsation[a]
	3/6:	blanching/decreased pulsation[a]	
Ramsay (6)	7/8:	blanching/increased pulsation	
White dermographism	2/7:	blanching/decreased pulsation	
	5/7:	blanching/unchanged–slightly increased pulsation	

[a] Somewhat varying values.

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DISCUSSION

Soter (Boston). Q: Were morphologic studies done to look at the state of vessels?

A: No such investigations were performed.

Voorhees (Ann Arbor). Q: Are there any differences between patients with and without cold fingers, by these methods?

A: By pulse plethysmography, earlier data on depressed temperature/blood flow could not be confirmed, though these data are preliminary, and I would be glad if you have found differences in your laboratory.

Soter (Boston). Q: Have you looked at variations on the same subjects?

A: Yes, the same patients may not react every time in the same manner. Instead of vasodilatation/constriction, lability of vessels may be stressed.

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COMPUTER ANALYSIS OF NOCTURNAL SCRATCH IN ATOPIC DERMATITIS

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Abstract. Scratch behavior was monitored with a paper strain gauge attached to the dorsum of the hand and an amplifier. The all-night recording was subsequently analysed every 60 sec with a specially devised computer system and the duration of scratching and the time(s) of its occurrence were printed out. This technique was applied in 10 patients with moderate or severe atopic dermatitis, 5 with generalized eczema and 5 non-itchy subjects. It was found that scratching occurred in cluster(s) or in an isolated form in both itchy and non-itchy subjects. Marked clustering of scratch behaviour was noted only in itchy subjects. No specific difference was observed in scratch patterns of atopic dermatitis vis-à-vis generalized eczema. Total scratch time of the right and left hands during the night ranged between approx. 1 and 5 thousand sec in itchy patients and between 1 and 5 hundred sec in non-itchy subjects. The ratio of the total scratch time to the recording time in atopic dermatitis did not differ significantly from that in generalized eczema. No correlation was observed between the duration of scratch and serum IgE level.

Key words: Scratch; Nocturnal scratch; Itching; Atopic dermatitis; Computer analysis

It is widely accepted that atopic dermatitis (AD) is a disease of itchiness, while scratching plays a cardinal role in the formation of characteristic skin lesions. However, itching occurs in many other skin diseases and the specific role played by scratching in the pathogenesis of AD has not been clarified. A previous study (1) revealed that scratch occurred in all stages of sleep (awake, 1, 2, 3, 4 and REM) without apparent difference between AD and the other itchy skin diseases.

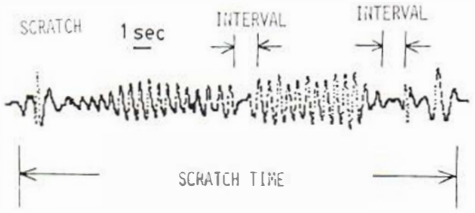

Fig. 1. Digitally signalled scratch waveforms and the definition of scratch (see text).

To facilitate the investigation of nocturnal scratching, we have developed a computer analysis system with which the all-night recording can be analysed relatively quickly. This was applied to AD, in comparison with generalized eczema (EZ) and non-itchy controls (CR).

SUBJECTS AND METHODS

Subjects

Three groups were studied (Table I):

I. Atopic dermatitis (AD). 10 Patients with persistent AD of adult type, all of whom had widespread skin lesions and who complained of moderate or severe itching.

II. Generalized eczema (EZ). Five patients with EZ of varying type and duration who complained of moderate or severe itching.

III. Non-itchy control (CR). Five non-itchy subjects including 2 normals and 3 patients with various diseases.

All-night scratch recording

Patients were usually admitted to the hospital on the day of

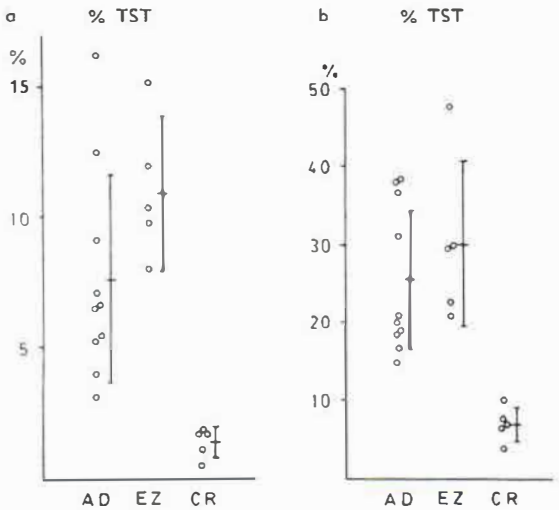

Fig. 2. Distribution of %TST (*a*) and %TSAT (*b*) in atopic dermatitis (AD), generalized eczema (EZ) and non-itchy control (CR). $\overline{+}$ indicates mean and ± 1 standard deviation.

Table I. *List of data*
For abbreviations see text

Group	Patient no, sex, age	Recording time (min)	TST (R + L) (sec)	% TST	TSAT (min)	% TSAT
Atopic dermatitis	1 F 43	560	5 435	(16.2)	173	(30.9)
	2 M 29	546	4 097	(12.5)	200	(36.6)
	3 F 16	521	2 842	(9.1)	199	(38.2)
	4 F 26	457	1 954	(7.1)	173	(37.9)
	5 M 26	547	2 159	(6.6)	113	(20.7)
	6 F 20	542	2 121	(6.5)	90	(16.6)
	7 M 22	560	1 811	(5.4)	105	(18.8)
	8 M 44	560	1 733	(5.2)	103	(18.4)
	9 M 15	519	1 242	(4.0)	103	(19.8)
	10 F 47	560	1 054	(3.1)	83	(14.8)
Generalized eczema	11 M 84	560	5 091	(15.2)	267	(47.7)
	12 M 54	546	3 915	(12.0)	162	(29.7)
	13 F 45	560	3 498	(10.4)	126	(22.5)
	14 M 47	418	2 467	(9.8)	123	(29.4)
	15 M 28	560	2 359	(7.0)	116	(20.7)
Non-itchy control	16 M 20	493	519	(1.8)	38	(7.7)
	17 M 37	485	500	(1.7)	48	(9.9)
	18 F 36	435	452	(1.7)	28	(6.4)
	19 M 19	503	345	(1.1)	34	(6.8)
	20 M 48	540	146	(0.5)	21	(3.9)

the test. A paper strain gauge was attached to the dorsum of the each hand by means of adhesive tape. This was connected to an amplifier (Nihonkoden RP5) and then to a data recorder (SONY UFR61430). Feasibility of the system was checked by a test scratch movement visualized on a display monitor. Recording was started between 9 and 10 pm and was finished around 6 am in the standard test.

Computer analysis

The record was analysed on a Digital Equipment Corp. PDP 11/40 computer with 28 KW of core memory, a magnetic disk (2.4 MW), a graphic display and an analog-to-digital conversion unit. The system was designed so that it can be operated at 16 times real time, making it possible to process a 9 hr scratch record in about 50 min. The analogous signal was filtered through the 1–5 Hz band pass filter and then converted to the digital form at an effective sampling rate of 20 Hz/channel and the sampled data were stored on a magnetic disk. The digital signal was processed every 60 sec and the analysis was made by the zero-crossing method combined with the amplitude and period criteria. The scratch was defined as the waveforms between 1 and 3 Hz with the amplitude exceeding a certain threshold level and with the duration greater than 2 sec. An interruption of less than 3 sec in between two scratches was counted as a continuous scratch (Fig. 1).

RESULTS

Difference between 1st and 2nd night

Six subjects (4 AD, 1 EZ and 1 CR) were tested on two nights and % total scratch times (see below) for the two nights were compared in each individual. An appreciable decrease was noted in 2 AD and 1 EZ and a marked increase in 1 AD on the second night. Almost identical results were obtained in 1 AD and 1 CR. All data described in this paper are those for the 1st night, except for a few examples.

% Total scratch time (%TST)

The duration of scratching throughout the night was summed for each hand and addition of the two hand records gave the total scratch time (TST). This was believed to indicate active scratching during the night. TST was divided by the recording time(RT) to give the %TST (Table I). %TST was compared between disease groups but the difference in the means was not significant for AD vs. EZ, while it was highly significant ($p < 0.001$, t-test) for AD and EZ vs. CR (Fig. 2*a*).

% Total scratch-associated time (%TSAT)

The minute in the record in which scratch occurred in either or both hands was summed up to give the total scratch-associated time (TSAT). This was divided by RT to obtain %TSAT which is thought to indicate the relative ratio of time occupied by

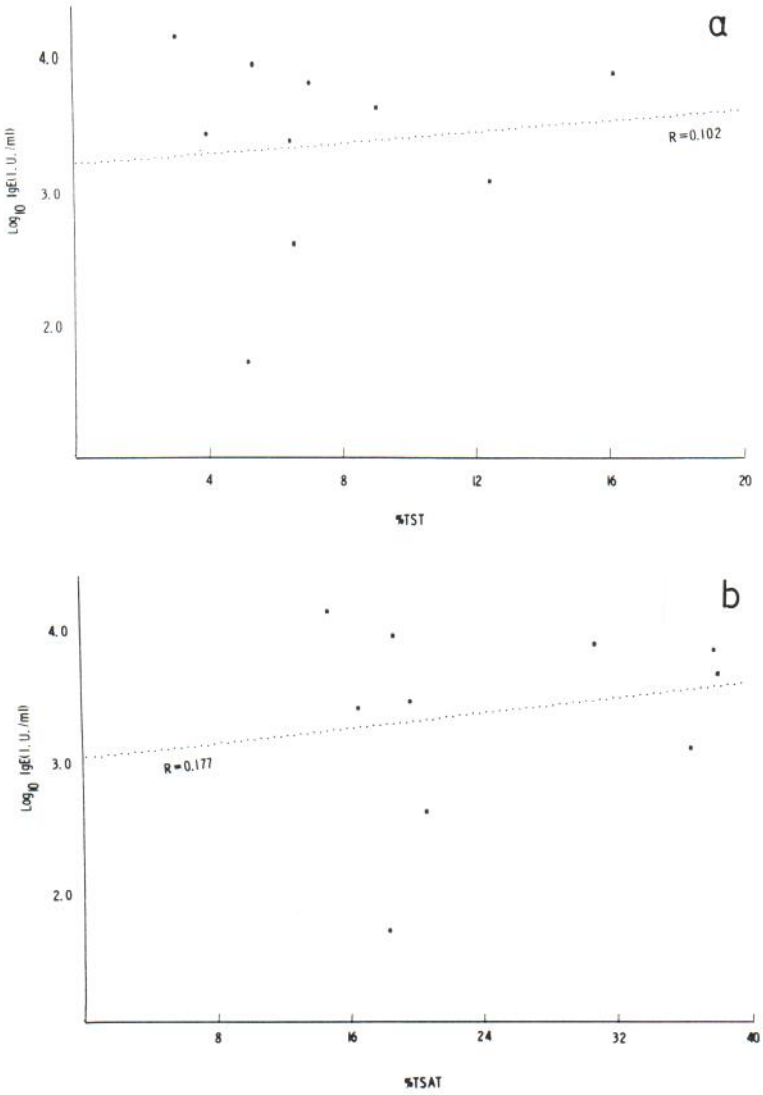

Fig. 3. Relation of $\log_{10}$ serum IgE to %TST (*a*) and %TSAT (*b*).

scratching (Table I). The difference in the means of %TSAT was not significant for AD vs. EZ, while it was highly significant ($p < 0.001$, *t*-test) for AD and EZ vs. CR (Fig. 2*b*).

Relation of scratch to serum IgE

%TST and %TSAT were compared with the log value of serum IgE in each AD individual. In 7 of 10 patients, IgE was estimated within a month of the test. As shown in Fig. 3*a* & *b*, no correlation was noted in either test.

Graphic display of scratch

The time occupied by scratching in every 60 sec was printed out on the ordinate (1 sec = 1 point) and the time of its occurrence on the abscissa. Two examples (AD no. 4 and CR no. 17) are shown in Fig. 4*a* & *b*. As seen in the figures, scratch occurred sometimes in a cluster and sometimes in isolated form. A cluster usually ranged over a few or several min. This may be called a small cluster. However, large clusters ranging over 20, 40 or even more than 60 min could also be observed. The large cluster was comprised of small clusters and isolated scratches. It appeared in more or less all itchy patients (AD and EZ), usually several times or occasionally during the night. In some it appeared in the early stages of the record and in others in the late stages. Scratches of the right and left hands often

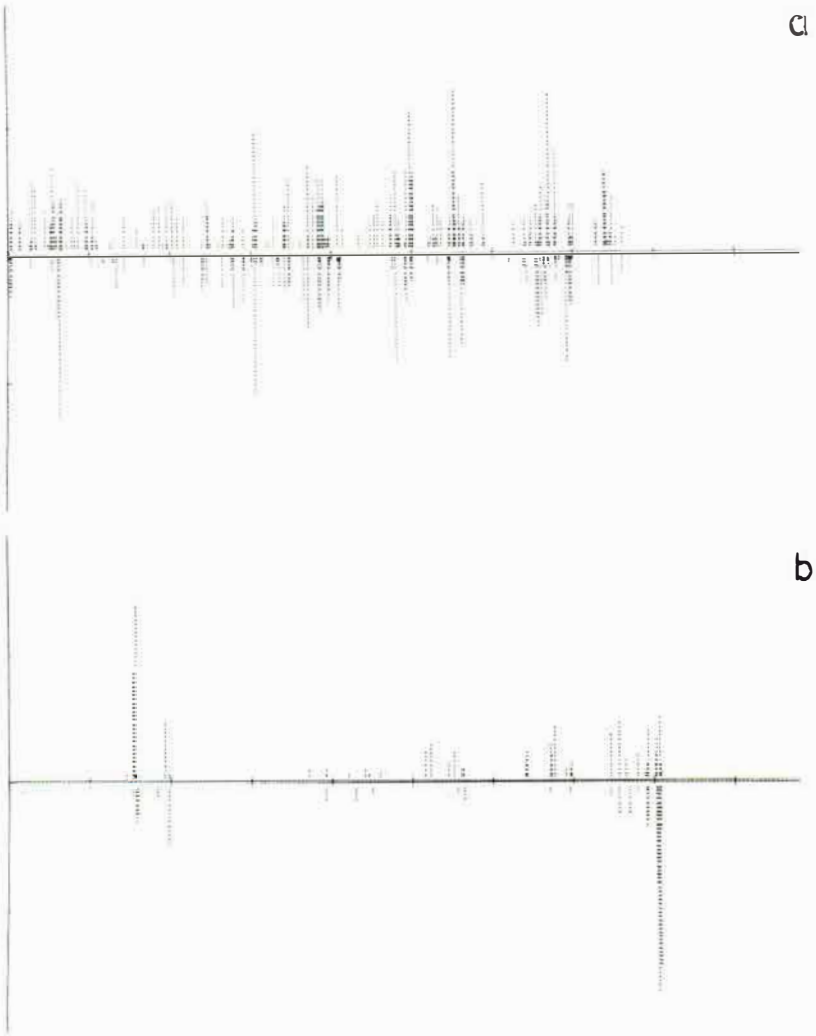

Fig. 4. Graphic display of nocturnal scratch. (*a*) Atopic dermatitis (no. 4), (*b*) non-itchy control (no. 17). Ordinate: time occupied by scratching in every 60 sec (1 sec = 1 point), upward, right hand and downward, left hand from the horizontal line of the middle. Abscissa: time flow from left to right (1 div = 1 h).

occurred at nearly the same time but alternative scratches were also noted on occasions.

There seemed to be no specific difference in the scratch patterns of AD and EZ.

A not insignificant amount of scratch was noted in CR. Most scratches were isolated sporadic ones, but a few small clusters were sometimes observed. The large cluster did not appear in any of the CR subjects.

DISCUSSION

Research into nocturnal scratching has not been an easy task. Savin et al. (1) initiated studies into nocturnal scratching in AD by all-night recording on paper and painstaking reading. Felix & Shuster (2) devised simple methods to estimate scratching during the night and applied them to the evaluation of the response to treatment. The computer analysis system described in this paper constitutes a reliable and quick method for the analysis of nocturnal scratch.

Graphic display disclosed that nocturnal scratching appeared in a cluster or in an isolated sporadic form in both itchy and non-itchy subjects. In very itchy patients, scratch formed several or a few large clusters, each of which lasted for 20, 40 or even more than 60 min. Since the human sleep cycle is approximately 100 min in length, repeated several times a night, large scratch clusters may be related to certain stages of sleep. Research into this subject is now in progress in our laboratories (3).

Patients with AD scratch much more during the night than during the daytime. This may be attributable to the increased body temperature or to the absence of distraction by daily activities (4). The relation of scratching to various physiological functions of the body is a subject of interest that will be elucidated in future investigations.

Many dermatologists believe that the skin lesions of AD are induced by scratching and we are inclined to accept as a matter of course that patients with AD scratch more often than those with the other types of eczema. This is symbolized by the general mention that polished nails are characteristic of AD. However, our results indicate that this does not seem to be true. The aggravating effect of scratch is a general phenomenon seen in various kinds of dermatitis and excema. It seems, therefore, that what is resposible for the formation of characteristic skin lesions in AD is not the scratching itself, but probably the preceding inflammation which renders the skin hyperresponsive to scratching in a specific way.

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DISCUSSION

Hanifin (Portland). Q: The data you presented referred to quantitative changes and I wonder if you have any information with regard to qualitative changes, and when the patients come in the hospital there is a miraculous resolution of the disease. Have you been able to follow these patients over a period of time in hospital and see if the itching continues?

A: With this method I cannot estimate any kind of quantitative character of itching. That must be studied separately

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ITCH AND IgE IN ATOPIC DERMATITIS

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Abstract. It was found that a correlation exists between the values of IgE in the serum and the duration of experimental itch elicited by trypsin in 20 patients with atopic dermatitis.

Key words: Experimental itching; Trypsin; IgE

The aim of the study was to investigate whether there is a correlation between itch and high IgE levels, since elevated serum/IgE was found in the majority of common dermatitides (1). In addition, it was assumed that persistent scratching can lead to a rise in the IgE level.

MATERIAL AND METHODS

20 adult atopic dermatitis patients with severe skin lesions were investigated. Determination of IgE in serum was performed by the usual PRIST method. Intensity of itch was determined by the duration of itch after intracutaneous injection of 0.1 ml trypsin 1:10 000 in the uninvolved skin of the arms (2).

RESULTS

According to Fig. 1 a positive or negative correlation existed between the level of IgE and the degree of experimental itch in atopic dermatitis patients.

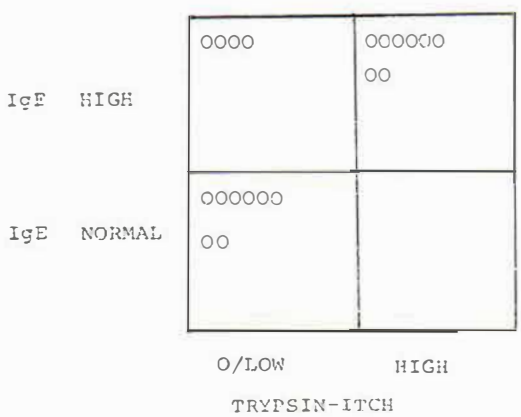

Fig. 1. Correlation between experimental itch and IgE. Open circles denotes patients with atopic dermatitis.

DISCUSSION

Experimental itch shows a fairly good correlation to clinical itching (2). According to the results, there is a parallel between high IgE levels and the main symptom of the disease, i.e. severe itch, in atopic dermatitis patients. It is not known whether there is a causal correlationship between IgE and itch, but presumably these two parameters of the disease merely reflect the severity and/or extent of the atopic dermatitis.

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DISCUSSION

Barnetson (Edinburgh). Q: Most patients with severe atopic eczema tend to have the highest IgE concentrations. But the concentrations remains pretty constant, whether they be in exacerbation or remission. This, I think, speaks against the thesis of correlation between itch and IgE.

A: My personal view is that IgE levels are not so constant, but this point was not investigated here.

Zachariae (Aarhus). Q. How does medication influence your assay?

A: The patients were not given medication during the study.

Voorhees (Ann Arbor). Q. Would it be feasible to inject IgE into normal individuals and atopic dermatitis patients to see if the injection of IgE might precipitate itching.

A: Many circumstances may affect such an experimental design.

Atherton (London). Q. How much are the levels of IgE influenced by the presence of other atopic diseases, particularly respiratory atopy?

A: The patients were pure atopic dermatitis subjects.

Q: In Italy we have an epidemic of helminthiasis in young children with atopic dermatitis. Is there any evidence of the same here in Scandinavia?

A: No helminthiasis was found in this material.

Aly (San Francisco). Q: In atopic dermatitis there is a large population of staphylococci and they are responsible for producing toxins and metabolites which can be itchy. In the literature there are indications that when the *S. aureus* population is reduced, itch is reduced.

A: I agree.

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EXPERIMENTAL ITCH AS A DIAGNOSTIC METHOD

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Abstract. Experimental trypsin itch was investigated in 100 patients with atopic dermatitis and in 115 controls. The duration of itch exceeded 2 minutes in 62.8% for involved skin and in 37% for uninvolved skin of atopic dermatitis patients, which are significantly higher percentages than in controls. The longer itch duration is not a specific feature of atopic dermatitis, however.

In numerous publications, Rajka has described the experimental itch duration method of intracutaneous application of trypsin in patients suffering from atopic dermatitis (AD) and other dermatoses. He found that the significantly longer itch duration in AD patients is a characteristic but not a specific feature.

The diagnosis "atopic dermatitis" is established in most of the cases according to the following criteria; personal and family history, clinical course, the sometimes elevated serum IgE level, white dermographism and the delayed blanch from cholinergic agents. It is certainly known that are some other kinds of eczema with clinical features similar to AD, but caused by different agents, such as contact and inhalative allergens. In our experience there are sometimes also types of chronic polymorphous light eruption in children with clinical features of AD. In this regard we have tried to differentiate those cases with AD-like feature by means of the lymphocyte transformation test and epi- and intracutaneous testing.

The itch duration determination method following intracutaneous application of trypsin, as described by Rajka appeared to us suitable as a complementary parameter in the differential diagnosis of borderline cases particularly between AD and other eczemas. To these one should also add the dyshidrotic types of AD which are localized on the hands.

PATIENTS AND METHODS

A group of 100 patients suffering from AD were compared with another group, of 115 subjects, 45 of them with various eczemas including seborrhoeic dermatitis, chronic recurrent urticaria, DLE, psoriasis vulgaris and candidiasis; 10 of them with acute gonorrhoea, 35 of them with internal diseases including malignant tumours and 25 healthy ones.

Intracutaneous application of 0.03 ml Trypure Novo in a concentration of 1:10 000, was carried out on the right forearm and 0.03 ml of normal saline as control on the left forearm. The latent time, i.e. the lag between the injection and the beginning of the itching, was in all cases 20–25 sec. The itch duration in 46% of the AD patients exceeded 2 minutes, while only 20% of controls had the same values.

The differences in scatter and Student's *t*-test were both significant. We found an itch duration of more than 2 min on the involved skin in 62.8%, while on the uninvolved skin in only 37% of the investigated AD patients. No statistical difference between severity degrees 0 to IV could be observed.

The itch duration in the healthy volunteers was without exception shorter than 2 min 10 sec and we therefore considered all values lower than that as normal. In this regard, only 43% of the AD patients showed prolonged itch duration. Finally we compared the itch duration in AD with other itchy skin diseases such as urticaria, various eczemas including nummular dermatitis and lichen planus.

There was a predominance of control dermatosis subjects among those with itch duration not exceeding 3 min, whereas among those with itch exceeding 3 min, AD patients predominated.

RESULTS AND DISCUSSION

Of statistical significance and as could be indicated according to our findings, an itch duration of more than 2 min following intracutaneous trypsin application speaks for but is not a specific feature of AD, as 23% of the controls (particularly 7 out of 20 patients with eczemas including seborrhoeic dermatitis) also showed the same itch duration. One could conclude accordingly that the trypsin test is helpful

but not decisive in the differential diagnosis of atopic dermatitis.

In order to strengthen our findings we proceeded with the same investigation with Dr Stark before and after a 6-week climatic cure at the Baltic Sea (Heiligendamm). During this period, an average shortening of the itch duration of about one minute in 150 patients with AD could be observed. The difference between initial and final values in psoriasis vulgaris and eczema vis-à-vis the low initial values was only about half. No patients received medication. Thus, the trypsin test could also be regarded as an objective method of measuring the effect of climatic cure.

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THE SIGNIFICANCE OF MORGAN'S FOLD IN CHILDREN WITH ATOPIC DERMATITIS

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Abstract. Among 40 children with atopic dermatitis between 1-4 years 5 had Morgan's fold, whereas none among 40 controls. Among 60 children with atopic dermatitis with an age average of 8 years 4 had this sign and in matched control series 2 children.

Key words: Morgan's fold; Atopic dermatitis

In an age of ever increasing sophisticated laboratory tests and a computer type approach to patients, clinicians are more and more tempted to omit a physical examination. Clinical observation has become almost a lost art and many doctors allow their diagnostic powers to atrophy. Over the years many physical signs have been described as characteristic of various diseases. Some of these signs have vanished without trace but others have stood the test of time. It must be admitted that they cannot overrule the objective result of a measurable laboratory test. Nevertheless they should not be forgotten and the complete clinician, especially dermatologists, should always be ready to practice and sharpen their powers of observation.

In 1948 according to Morgan, Charles C. Dennie called the attention of his students and associates to the sign which he had observed to be rather pathognomic in most cases of allergy especially in persons with a history of eczema, hay fever, and asthma. This sign was a definite wrinkle just beneath the margin of the lower lid of both eyes. It is usually present at birth or shortly thereafter. It is retained through life. Rajka in his monograph on atopic dermatitis also draws attention to an infra-orbital crease similar to that seen in mongolism. He states that it can usually be observed in atopic dermatitis; it may occur more frequently in certain ethnic groups. Two modern text books also describe the sign. Weinberg, Leider and Shapiro in their illustrated textbook on paediatric dermatology illustrate a child with atopic dermatitis with what they describe as characteristic deep grooves in the lower eye lids. Moschella, Pillsbury, and Hurley in their textbook on dermatology have also an illustration of a double fold under the eye which they state illustrates Morgan's fold, Dennie's fold or Mongolian fold. Thus the sign has been varyingly described as a wrinkle, crease, groove, and fold.

PATIENTS AND METHODS

It was decided to examine a series of children suffering from atopic dermatitis to find out the prevalence of the sign. Children between the age of 5 and 15 were examined. These children had a history of recurrent atopic dermatitis for a couple of years. There were 60 children in this series, 30 girls, and 30 boys, average age 8. At the same time a control series consisting of 60 children suffering from warts and with no history of atopy were examined. There were 37 girls and 23 boys, average age 9.

It is inevitable that in the evaluation of a clinical sign subjective impressions and bias must play an important part. In a clinical sign that depends on the presence of folds of skin and furrows, there must be gradations and variations. On examination of the children it was quickly apparent that some children showed rudimentary furrows and folds. Therefore, the problem arose as how marked had the furrows and folds to be before being classified as a positive sign.

It was decided that only children who demonstrated a definite double fold as illustrated in Moschella, Pillsbury, and Hurley's textbook should be taken as positive.

There were four positive cases in the series of atopic dermatitis and two positive cases in the control series. There was no correlation between severity of dermatitis and the presence of a definite wrinkle. There was also no history of rubbing of the eyes. In the atopic series 2 of the positive cases were boys and 2 were girls, and in the control series the 2 positive cases were boys.

Next forty children between the ages of one and four years with active atopic dermatitis were examined. In this series there were 21 males and 19 females with an average age of 1 year 8 months. A control series consisting of 40 children between the ages of one and four years were also examined, average age 1 year 5 months. There were 25 males and 15 females in the group. They were suffering from various diseases but none had an atopic condition. There were 5 positive cases, 3 boys and 2 girls in the atopic series and none in the control series.

RESULTS AND COMMENTS

It is not the purpose of this short communication to present any definite results, the figures are too small to come to any conclusion. Possibly cautious comment may be made on some aspects. It is interesting that the sign appears to be more prevalent in the younger atopic age group. Indeed some further cases examined but not included in this series confirm this trend. It poses the question of whether the sign can disappear as the atopic child grows older. Further follow up studies are necessary.

The purpose of this paper is rather to recall to mind Morgan's fold and to make a plea for retention of our clinical expertise. Perhaps truth like beauty is in the eye of the beholder, or in this case in the eye of the patient.

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DISCUSSION

Vickers (Liverpool). Q: It takes 15–20 years for most of Morgan's folds to disappear. Is it an abnormality in the eyelid or is it eczema localised to this area producing this double fold?

A: I never dared to take a biopsy. I agree with Dr Vickers. I would not dream of taking a biopsy in any of these cases. There is no correlation between the severity of the atopic dermatitis and there is no definite history of rubbing of this lesions, which is another possibility in keeping the fold growing by rubbing. I think possibly that it is associated with that rather rough pale skin which was characteristic of the atopic condition.

Atherton (London): I never saw Morgan's fold in any baby at all who did not have eczema.

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NATURAL KILLER CELLS AND INTERFERON PRODUCTION IN ATOPIC DERMATITIS

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Abstract. Natural killer (NK) cells, cytotoxic for Burkitt lymphoma cells, were found to be more active in atopic than in non-atopic children. Upon stimulation of lymphocyte cultures with Sendai virus, less interferon was produced by cells from atopic than from non-atopic individuals. Increased NK activity and decreased interferon production may both be consequences of a T lymphocyte deficiency which has been suggested to be causally related to atopic disease.

Key words: Natural killer cells; Sendai virus; Interferon; Atopic disease

Recently, evidence has accumulated that atopic allergy is associated with a T lymphocyte deficiency (reviewed in ref. 11). Although it has not been definitely excluded that this deficiency is secondary to treatment or to manifestations of the disease, this seems unlikely for two reasons; first, low T cell counts and decreased lymphocyte responsiveness to mitogens and antigens are obvious even in patients without overt disease and without treatment (5) and second, low T cell counts are present in babies of atopic parents in early life, before atopic disease and elevated levels of IgE develop (7).

The consequence of a T cell deficiency is certainly dependent on the degree and quality of depression of various T cell functions. In atopy there is some evidence that suppressor T cell function is abnormally low (10) and that suppressor cells regulating, in particular, IgE synthesis are deficient (1). Thus one of the consequences of the T cell deficiency may be hyperproduction of IgE. However, the findings of depressed delayed hypersensitivity reactions to various antigens and the increased susceptibility of atopics to severe infections with certain viral and fungal agents (reviewed in ref. 2) certainly suggest that several different T cell functions are disturbed in atopy.

In the present paper we suggest two mechanisms by which the cellular defect in atopy may lead to disease. First, several immunological functions are subject to regulation by suppressor cells. For instance, the activity of cytotoxic, so-called natural killer (NK) cells may possibly be regulated by suppressor cells (9) and failing suppressor function might therefore lead to hyperactivity of NK cells and hence disease. Second, several kinds of immunocompetent cells appear to have the ability to produce interferon (12). A faulty function of some particular kind of lymphoid cell might therefore, directly or indirectly, result in depression of interferon (IF) production and hence to increased susceptibility to viral infections. In this paper we present data on NK cell activity and IF production in children with atopic disease.

PATIENTS AND METHODS

Patients. Blood specimens were obtained from children with asthma, rhinoconjunctivitis and/or atopic dermatitis, who visited the outpatient clinic at the Childrens Hospital, Göteborg. To be included in the study the children had to have positive skin tests and elevated serum levels of specific and/or total IgE. Control specimens were obtained from healthy children without history or signs of atopy.

Assay of natural killer cell activity. The target cells used (Burkitt lymphoma cells of the P3HR1 line) were labelled with Cr^{51} for 4 hours, then washed and placed in microtrays. Lymphocytes, prepared by centrifugation in Metrizoate-Ficoll, were then added to the target cells at a ratio of 25:1. After 18 hours' incubation at 37°C, aliquots of the supernatants of the cultures were collected, and the radioactivity determined in a gamma counter. Specific lysis was calculated as:

$$\frac{\text{cpm in test sample} - \text{cpm in medium control}}{\text{cpm in totally lysed sample} - \text{cpm in medium control}} \cdot 100$$

Assay of interferon production. Metrizoate-Ficoll separated lymphocytes were cultured in microtrays at a cell concentration of 1.5×10^6/ml. Sendai virus at a final concentration of 300 hemagglutinating units per ml was added to the cultures. After incubation for 4 days the culture supernatants were frozen and then titrated for IF content essentially according to the method of Havell & Vilcek (3). The cell used for titration were of bovine origin (MDBK cells) and as challenge virus vesicular stomatitis virus was used.

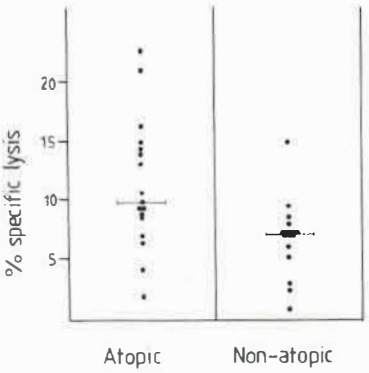

Fig. 1. Natural killer cell activity againts P3HR1 cells in atopic and non-atopic children. Studies performed on 17 atopic (mean age 11.7) years and 16 non-atopic (mean age 12.2 years) 9–17-year-old children. Effector : target cell ratio was 25 : 1. Horizontal bars indicate median values. The difference between the two groups is statistically significant (Wilcoxon test, $p < 0.01$).

The titres obtained were calibrated against an international reference IF preparation.

RESULTS

Natural killer cell activity. The NK activity against P3HR1 cells was tested in 51 1–17-year-old children (19 controls and 32 with various atopic diseases). There was no evident age dependency of the activity. Thus the median activity (% specific lysis) in the group of atopic 1–8-year-old children was 10.2% and the median activity in atopic 9–17-year-old children 9.7%. In the whole material NK cell activity was significantly higher in atopic than in non-atopic children (Wilcoxon test, $p < 0.01$). The same significant difference ($p < 0.01$) was found when only the age group 9–17 years (17 atopic and 16 non-atopic children) was considered (Fig. 1). The number of non-atopic children in the age group 1–8 years was too small to allow statistical calculations.

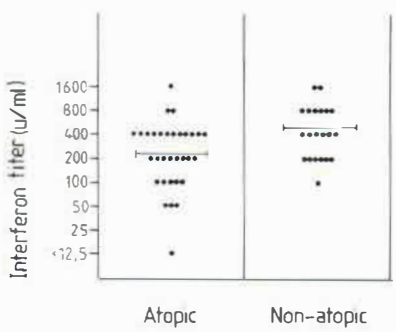

Fig. 2. Interferon production in lymphocyte cultures from atopic and non-atopic children. Studies performed on 32 atopic (mean age 11.8 years) and 21 non-atopic (mean age 12.3 years) 9–17-year old children. Interferon titers were determined after 4 days of culture of Sendai virus-stimulated lymphocytes. The difference between the two groups is statistically significant (Student's *t*-test, $p < 0.01$).

Interferon production. The production of leukocyte IF in lymphocyte cultures stimulated with Sendai virus was studied in 45 atopic and 21 non-atopic 1–17-year-old children. There was a tendency towards age dependency of IF production, since the median values obtained in 1–8-year-old atopic children were somewhat higher than those obtained in the atopic 9–17-year-old individuals. The difference, however, was not statistically significant. When the IF-producing capacity of age-matched children was studied, it was evident (Fig. 2) that the cells from atopic individuals had a depressed capacity to form IF upon stimulation with Sendai virus (Student's *t*-test, $p < 0.01$). One of the atopic children did not produce any detectable IF. This child had a very severe disease, with asthma, eczema, very high serum IgE levels and low T cell counts. Apart from this case there was no obvious correlation between severity of disease and ability to produce IF.

DISCUSSION

During recent years speculation on the pathogenesis of atopic diseases has been focused on the hyperproduction of IgE, which is frequently associated with asthma as well as with atopic dermatitis (6). Many cases of atopic dermatitis have perfectly normal levels of serum IgE, however, and it is hard to envisage the histological picture in the skin of dermatitis patients as exclusively being caused by IgE-mediated release of vasoactive mediators.

The hyperproduction of IgE has earlier been suggested to have a causal relationship to a T cell deficiency and a possible chain of events leading to increased IgE-antibody formation has been proposed (11). If there is, as reported, a more pronounced deficiency in some T cell subsets, notably suppressor cells, than in other cell populations, consequences apart from increased IgE-production may be anticipated. In our present study we have obtained evidence that one of these possible consequences, namely hyperactivity of cytotoxic cells, may in fact occur in atopic disease. Although our experimental set-up did not definitely exclude the possibility that antibody-dependent cellular cyto-

toxicity rather than NK activity was being measured, the results suggest that NK cell activity is increased in atopy. The relevance of this finding for the pathogenesis of atopic dermatitis remains to be elucidated.

Another consequence of defective T lymphocyte function in atopy that can be envisaged is decreased production of various lymphokines. Recently Horsmanheimo et al. (4) have presented evidence that the production of leukocyte migration inhibitory factor is subnormal in atopic patients. In the present study we found that stimulation of lymphocyte cultures with Sendai virus resulted in slightly subnormal IF production in atopic individuals. The experimental procedure was designed to measure production of classical leukocyte IF rather than so-called immune IF and it is therefore unclear to what extent the decreased IF production is related to abnormal T cell function. Studies are in progress to determine the production of immune IF, which is clearly T cell-dependent (8) in atopic individuals.

Since the amount of IF produced was less in atopics than in non-atopics, the present findings may have a bearing on the pathogenesis of atopy. It is well known that some viral infections, e.g. those caused by vaccinia and herpes simplex virus, may run an unusually severe course in atopics and also that warts are very common in atopics (reviewed in ref. 2). It is tempting to speculate that our finding of decreased IF production in the atopic has some bearing on the course of these viral diseases. It is possible, however, that intrinsic skin abnormalities are of greater importance for the spread of these diseases, since there is no evidence that atopics have a generally increased susceptibility to viral infections.

ACKNOWLEDGEMENTS

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DISCUSSION

Zachariae (Aarhus). Q: What do you think about prophylaxis, i.e. antigen avoidance, in atopic children?

A: It is well known in several systems that if you give large amounts of a mitogen or an antigen you get an activation preferentially of suppressor cells. On the other hand there are data showing that antigen elimination is of definite value for the prevention of atopic disease so I think it's a tough question.

Hanifin (Portland). Q: How old were the children you investigated and was there a difference between cow's milk versus breast milk-fed?

A: The T cell levels were determined at one month of age and serum IgE levels at 6 months of age. Children with asthmatic fathers had the lowest T cells, and the only definite correlation between low T cell values and high IgE values was obtained in the children that were fed cow's milk.

Saurat (Paris). Q: You saw increase lymphocyte cytotoxicity in atopic dermatitis, was it direct cytotoxicity?

A: Yes this was natural killing. We used cells bearing EB-virus and cannot be absolutely sure that one is not measuring antibody-dependent cellular cytotoxicity.

Giannetti (Pavia). Q: What is your opinion about Tγ and Tμ markers? and did you look at IgE levels?

A: In one investigation the number of Tγ cells was decreased in atopy, but several others did not find any difference between normals and atopics. A defective suppressor function of macrophages should also be considered. We have not studied the effect of suppressor cells on IgE production.

Thestrup-Pedersen (Aarhus): We have investigated 16 adults with atopic dermatitis and found a change in the ratio between the Tμ and Tγ cell, viz. a decrease in Tγ cells and a slight increase in the Tμ cells.

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CHEMOTAXIS INHIBITION BY PLASMA FROM PATIENTS WITH ATOPIC DERMATITIS

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Abstract. We previously reported depressed polymorphonuclear leukocyte and monocyte chemotaxis in patients with severe atopic dermatitis. The degree of impairment roughly correlated with the disease severity and chemotaxis was noted to improve rapidly with clinical remissions. This rapid improvement suggested the presence of a short-lived plasma inhibitor of leukocyte function. We used a radiolabeled PMN chemotaxis assay to assess plasma effect on migration. PMN's were incubated in plasmas from patients with varying degrees of atopic dermatitis then washed and assessed for migration toward endotoxin-stimulated serum attractant. Inhibitory effect varied widely and correlated directly with clinical extent and severity of dermatitis. Serial studies on individual patients showed lessening of plasma inhibitory activity during remissions. PMN's from patients with atopic dermatitis showed improved migration after incubation in normal plasma. There was no evidence for circulating chemoattractants nor chemotactic factor inactivators. There appears to be a circulating inhibitor of chemotaxis present in plasma during acute flares of atopic dermatitis; the molecular nature of the inhibitor remains to be elucidated.

Key words: Polymorphonuclear cell chemotaxis; Atopic dermatitis

Individuals with atopic dermatitis (AD) have depressed cell mediated immunity (CMI) (7, 10, 11) and increased susceptibility to viral and staphylococcal cutaneous infections (4). We have reported that polymorphonuclear leukocyte (PMN) and monocyte chemotaxis, as well as lymphocyte response to phytohemagglutinin (PHA) were simultaneously depressed in patients with atopic erythroderma (11). PMN chemotaxis was noted to improve rapidly with clinical remissions. Dysfunction of three major host defense cells during erythrodermic flares may contribute to the symptomatology and cutaneous infections of atopic dermatitis. These transient functional deficiencies of PMN's, monocytes and lymphocytes may provide basic insight into the relationship between inflammation and immunity.

Numerous abnormalities of human leukocyte chemotaxis have been described, including intrinsic cellular defects, deficient chemotactic factor production, humoral inactivators of chemotactic factors and humoral inhibitors of leukocyte migration. Snyderman et al. (13) have reported inhibition of normal monocyte chemotaxis by serum from severe AD patients. In this communication we report studies showing that plasma from patients with AD inhibited chemotaxis of normal PMN. Plasma inhibitory activity correlated with the severity of dermatitis and there was no evidence for chemotactic factor inactivators.

MATERIALS AND METHODS

The diagnosis of AD in patients was based on a constallation of typical clinical features, and extent and severity of skin involvement were graded on scales of 1 to 5 as previously outlined (11). Thus, a patient with generalized erythroderma would carry a rating of 5/5. Patient ages ranged from 3 to 62 years; most were young adults. Some of the patients were under treatment with systemic antibiotics when studied, but none were receiving corticosteroids, antihistamines or other agents. None of the patients had evident systemic disease. Control subjects were normal, healthy, young adults receiving no therapy.

Preparation of leukocytes

Heparinized venous blood was centrifuged on a Hypaque-Ficoll gradient and mononuclear cells were removed. Cells below the gradient included PMN's and erythrocytes (RBC's). The RBC's were sedimented with 3% dextransaline; residual RBC's in the PMN-rich supernate were then osmotically lysed. The remaining PMN's were suspended at 2.6×10^6/ml in Gey's solution containing 2% bovine serum albumin (GBSS) then subjected to the migration assay detailed below.

PMN chemotaxis assay

The isotope-labeled PMN chemotaxis assay of Gallin et al. was used in all studies. (Briefly, this assay measured the migration of ^{51}Cr labelled PMN's through one filter and onto a lower filter (2).) Radioactivity was quantitated using a gamma counter. All samples were run in triplicate; results were expressed as average counts per minute of three lower filters corrected for labeling variability between different donor cell populations as follows:

$$\frac{\text{Mean CPM of 3 filters} \times 10\,000.}{\text{CPM per } 10^6 \text{ PMN}}$$

This value was designated corrected counts per minute (CCPM).

Preparation of chemoattractant

Two ml normal AB or atopic serum, 1 ml sterile, *E. coli* lipopolysaccharide (Difco; 250 mcg/ml saline), and 17 ml veronal buffered saline were mixed and incubated at 37°C for 60 min followed by 56° for 30 min. The chemoattractant was then diluted with equal parts of veronal buffered saline and small aliquots were stored at −70°C until used.

Preparation of plasma and serum

Heparinized plasma was drawn into plastic syringes and immediately spun in plastic centrifuge tubes at 2 000 g for 30 min in at 4°C, then stored at −70°C until used for incubation studies.

Before addition to chemotaxis chambers, isotope-labeled PMN's were suspended in 1.0 ml plasma or serum (7.8×10^6 cells/ml) for 90 min at 37°C. The incubation tubes were periodically agitated and after 90 min the cells were washed once and resuspended in 3 ml GBSS. This cell suspension was then placed in the upper compartment of the chemotaxis chamber and migration was quantitated as described.

RESULTS

Effect of atopic plasma on normal PMN chemotaxis

In all studies the migration of normal PMN's after incubation in atopic plasma was compared with parallel migration of cells incubated in pooled, normal AB plasma. The same standard attractant was used in all cases.

Initial studies tested plasmas obtained from four patients during acute exacerbations of generalized atopic erythroderma (i.e. extent/severity ratings of 5/5). As shown in Table I, inhibition ranging from 42% to 82% was observed when the cells were incubated in plasma from these severely involved atopic dermatitis patients. Pilot studies compared chemotaxis of neutrophils incubated for 15, 20, 60 and 90 min in these plasmas and inhibition was found to be maximal between 60 and 90 min. The 90 min incubation period was used for all subsequent studies. In no study did Trypan blue or phagocytic tests show decreased viability (nor was either gross or microscopic aggregation of cells noted after incubation in atopic plasma.)

Table I. *Inhibition of normal PMN chemotaxis by plasma from patients with erythrodermic atopic dermatitis (mean CCPM ± S.E.M.)*

Normal plasma	Atopic plasma	Depression (%)
2 846 ± 309	1 675 ± 175	42
3 061 ± 28	629 ± 31	79
4 817 ± 439	829 ± 256	82
2 788 ± 525	1 184 ± 309	58

Table II. *Comparison of clinical extent and severity of atopic dermatitis with atopic plasma inhibition of normal PMN chemotaxis*

Based on extent/severity grades of 1–5

	Chemotaxis inhibition		
	≤ 20%	> 20%	*p*-value
Mean extent	2.8	4.1	< 0.001
Mean severity	2.3	3.8	< 0.001

We subsequently performed 91 studies from 28 patients with mild to severe atopic dermatitis. Cells preincubated in atopic plasma showed a mean migration of 1 845 CCPM (±S.D. 992) compared with 2 149 CCPM (±S.D. 1046) after normal control plasma. A *t*-test for correlated data showed these differences to be significant with a *p*-value of < 0.001.

Inhibitory activity of plasmas varied considerably with the clinical severity of disease. Extent and severity ratings were recorded for comparison with chemotactic inhibition by 30 atopic plasmas. Statistical comparisons of the mean extent and severity ratings of the 16 plasmas causing less than 20% vs. the 17 plasmas causing greater than 20% inhibition showed significant differences for both parameters (Table II). There clearly appears to be a correlation between clinical severity of atopic dermatitis and presence of a plasma chemotactic inhibitor.

Effect of normal plasma on atopic cells

Since incubation in atopic plasma could decrease normal PMN chemotaxis, we next investigated the effect of incubating atopic cells in normal plasma to reverse the inhibition. For these studies, chemotaxis of freshly isolated PMN's was compared to that of the same cells which had been incubated for 90 minutes in normal plasma. PMN's from 7 of 8 patients with atopic dermatitis showed increased migration after exposure to normal AB plasma (Table III). The mean increase from 1 482 to 2 239 CCPM was significant with $p < 0.001$ by paired comparison *t*-test.

Table III. *Effect of normal plasma pre-incubation on atopic PMN chemotaxis (mean CCPM ± S.E.M.)*

Exp. no.	Atopic cells Non-pre-incubated	Atopic cells after normal plasma	% Δ
1	3 035 ± 102	3 034 ± 85	0
2	1 802 ± 66	2 692 ± 336	+ 33
3	353 ± 85	1 396 ± 78	+ 75
4	1 185 ± 41	1 927 ± 70	+ 39
5	887 ± 52	1 441 ± 54	+ 38
6	1 303 ± 122	1 710 ± 34	+ 24
7	496 ± 28	1 225 ± 99	+ 60
8	2 799 ± 351	4 489 ± 280	+ 38
Mean	1 482 ± 331	2 239 ± 369	+ 38

Parallel control studies of normal cells in normal AB plasma showed no change in migration.

Chemoattractant activity of non-stimulated atopic plasma

We questioned whther the cellular inhibition observed with atopic plasma was due to random stimulation of neutrophils by chemotactically active molecules. To evaluate inherent chemotactic activity, we measured normal PMN migration using as attractants undiluted inhibitory plasmas obtained from 11 patients with severe AD. Migration varied widely but we were unable to detect significant chemoattractant activity of atopic plasmas compared with normal plasmas (Table IV). In one study normal plasma caused significantly greater migration, but this was not a consistent finding.

Effect of atopic serum on standard attractant

To detect the possible presence of circulating chemotactic factor inactivators, we mixed fresh serum from patients with AD 1:10 with standard attractant (endotoxin-stimulated normal serum). Chemoattraction of this mixture was then compared with

Table IV. *Comparison of normal and atopic plasmas as chemoattractants (mean CCPM ± S.E.M.)*

Figures in parentheses represent number of plasma tested in each experiment

Exp. no.	Normal plasma	Atopic plasma	*p*-value
1	(1) 1 270 ± 70	(5) 1 908 ± 294	< 0.1
2	(1) 1 639 ± 98	(2) 2 712 ± 327	< 0.2
3	(2) 4 335 ± 83	(3) 3 579 ± 419	< 0.3
4	(1) 2 850 ± 42	(1) 1 472 ± 25	< 0.001

Table V. *Effect of atopic serum on standard attractant*

Exp. no.	Attractant plus 10% normal serum	Attractant plus 10% atopic serum
1	2 791 ± 71	2 732 ± 73
2	2 623 ± 81	2 883 ± 68
3	3 974 ± 118	4 060 ± 51

10% normal control plasma in standard attractant. Results (Table V) showed no evidence of chemotactic factor inactivation by atopic serum.

DISCUSSION

Multiple basic defects have been identified to account for the various human leukocyte chemotaxis deficiencies. Abnormalities may be intrinsic to the cell or may be due to external influences directed at either the cell or the chemoattractant (12). In our studies of patients with AD we noted that neutrophil chemotaxis tended to be only transiently abnormal. Patients with depressed PMN migration during severe flares of dermatitis showed nearly normal chemotaxis 48 hours later, as their erythroderma subsided in the hospital (11). We hypothesized that a transient circulating factor may be associated both with leukocyte abnormalities and with cutaneous exacerbations.

This report documents inhibition of normal neutrophil chemotaxis by plasma from patients with AD. Inhibition was maximal when cells were incubated for 90 minutes prior to being washed and placed in the upper compartment of a chemotaxis chamber. As with the direct chemotaxis studies of atopic cells (11), the magnitude of plasma inhibition varied directly with the clinicial severity of disease. Plasmas obtained during remissions generally failed to cause significant inhibition.

Since cells had diminished chemotactic function after exposure to atopic plasma, we questioned whether the defect was reversible. Migration of cells from patients with AD significantly increased after incubation in normal plasma while normal control PMN chemotaxis did not increase. These findings indicate that atopic PMN's are not intrinsically defective, but are reversibly inhibited by a factor acting either on the internal metabolism of the cell or on the surface membrane. A previous study of chemotactic defects in the hyperimmunoglobulinemia-E syndrome failed to restore chemotactic responsiveness when cells were incubated in normal plasma

for 30 min (4) but in a later publication by the same authors, normalization of migration was observed when patient's blood was kept at room temperature for 12 hours before cells were isolated (5). Possibly because of differing migration assays, we noted reduced chemotaxis of both normal and atopic PMN's when we attempted to reproduce those findings.

The molecular nature of the atopic plasma inhibitor has not been characterized. We used cell and platelet-free, undiluted heparinized plasma for PMN preincubations throughout this study. Preliminary studies showed that preparation of blood with compounds such as trasylol, EACA and hexadimethrine bromide, to prevent activation of potentially chemotactic molecules in plasma did not offer any advantage. Heparinized plasma prepared in plastic equipment consistently supported greater migration than plasma plus these additives. Significantly less migration was seen with cells preincubated in serum. Incubation of cells in plasma-free media caused marked depression of chemotaxis as did manipulation of plasmas with heating or dialysis.

Decreased leukocyte chemotaxis has been associated with increased serum chemotactic factor inactivator in anergic patients with Hodgkin's disease (16), sarcoidosis (9), and cirrhosis (14). Our studies indicate that the atopic plasma inhibitor acts directly on cells rather than on chemoattractant. The cells are washed before transferring to chemotaxis chambers; it seems unlikely that enough of the factor could be transferred on cell membranes to cause inactivation of the standard attractant in the lower compartment of the chamber. We further investigated this possibility by directly combining atopic sera with our endotoxin-stimulated serum attractant and found no reduction in chemoattraction.

We also postulated that cells might be "inactivated" by circulating chemotactic factor. Chemotactically active molecules have been shown to cause random stimulation of PMN's, decreasing their directed migration (15). There was no difference in random migration between cells incubated in normal plasma and those incubated in atopic plasmas. Furthermore, we were unable to demonstrate chemoattractant activity with untreated atopic plasma.

Hill & Quie (4) have studied PMN chemotaxis in patients with hyperimmunoglobulinemia-E syndrome consisting of recurrent cutaneous infections, high levels of serum IgE and eczema. Serum from these patients did not inhibit chemotactic activity of normal PMN's. Since IgE is known to mediate histamine release, they suggested this agent might be responsible for the abnormal chemotaxis. They showed *in vitro* inhibition of PMN chemotaxis by histamine concentrations ranging from 10^{-3} to 10^{-5} M (4).

We have been particularly interested in this possibility because histamine could explain many of the abnormalities associated with atopic dermatitis, including cutaneous erythema and depressed lymphocyte responsiveness in addition to inhibition of chemotactic response. In unpublished studies, we have found elevated histamine levels in three atopic plasmas which caused inhibition of normal PMN chemotaxis; this has not, however, been a constant finding. Our observations of the cyclical flares of dermatitis in these patients suggests that histamine may be only briefly present before it is cleared or inactivated. Consistent documentation of a correlation between plasma histamine levels and chemotactic inhibition, therefore, would be difficult.

Histamine levels are elevated in the skin of patients with AD, and one might suspect that chemotaxis through involved skin might be decreased. We have used skin window preparations to demonstrate significant reductions in total cells and in the PMN to mononuclear cell ratio on the coverslips after three hours. Migration appears to be merely slowed, since differences between normals and atopics is negligible after 6, 12 and 21 hours (S. Grewe and J. M. Hanifin, unpublished data).

Thus, our *in vitro* findings appear to reflect an *in vivo* abnormality. This slowed migration through skin may well account for cutaneous anergy in patients who have normal lymphocyte transformation responses to microbial antigens (11).

Certainly, there are innumerable factors which could be responsible for the impaired chemotaxis in patients with atopic dermatitis. At this point we can only say that there is a circulating inhibitor of chemotaxis; this inhibitor is most active during acute flares of dermatitis and the exact nature of the inhibitor remains to be elucidated.

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ANTI-IgE INDUCED HISTAMINE RELEASE FROM BASOPHILS IN CHILDREN WITH ATOPIC DERMATITIS

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Abstract. Histamine release (HR) induced by anti-IgE (1 to 10 000 ng/ml) from whole-blood basophils has been evaluated in 29 children with atopic dermatitis (AD). HR was found to be reproducible and did not vary with the clinical improvement or aggravation of AD. Children with AD were found to have a higher HR than controls. Two groups of children with AD could be distinguished on the basis of histamine release. These groups did not differ with regard to age, age of onset, clinical severity, or serum IgE levels.

Key words: Histamine release; anti-IgE; Basophils

Histamine release from circulating basophils exposed to anti-IgE is one of the parameters used to test basophil "releasability". It seems likely that "releasability" could be an important factor for the understanding of allergic diseases (4). To our knowledge, "releasability" in infantile atopic dermatitis (AD) has not previously been studied. We have studied HR induced by anti-IgE in 29 children with AD, its spontaneous variability, and its correlation with various parameters.

MATERIAL AND METHODS

The release of histamine from circulating basophils was measured according to Siraganian (6); this technique is carried out with whole blood and not with washed buffy coat cells. Histamine release was assayed according to Shore (5) by means of a continuous flow automated fluorimetric method. The experimental error was ±2% for histamine concentrations in excess of 2 ng/ml and ±5% between 0.1 and 2 ng/ml. The results were linear between 0.1 ng/ml and 5 μg/ml. Anti-IgE serum was purchased from Sodelen and used at a concentration of 1, 10, 100, 1 000 and 10 000 ng/ml of anti-IgE antibody. Levels of serum IgE were measured by the immuno enzymatic method (2). This study concerned 29 children with atopic dermatitis (AD), mean age 4.2 years, ranging from 6 months to 14 years, each child being studied at different times. The activity of the disease was scored clinically according to the method of Clendenning (1). 18 normal children without a personal or a family history of atopy were tested as controls.

RESULTS

1. *Spontaneous variability of histamine release in AD.* Tables I and II show that when two HR determinations were performed at an interval of several weeks, in 28 children, only slight variations in HR were seen between the first, second and third determinations. These slight variations in HR were seen although greater (but not significant) variations in serum IgE levels were observed. Furthermore, there was a significant improvement in the clinical state without modification of HR.

2. *HR in AD and in controls.* Table III shows that a higher percentage of HR is induced by 1 000 and 10 000 ng/ml of anti-IgE in AD than in controls. This difference was not observed with the other concentrations of anti-IgE. Total histamine content was higher in AD than in controls.

3. *Subgroups in AD.* Eleven children were found to release less than 25% of the total histamine after

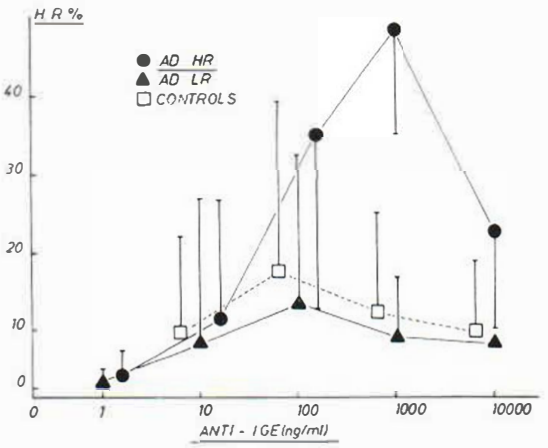

Fig. 1. Histamine release induced by varying doses of anti-IgE in normal children (control) and children with atopic dermatitis. ADHR = atopic dermatitis high responder, ADLR = atopic dermatitis low responder.

Table I. *Reproducibility of the histamine release induced by varying doses of anti-IgE*

The mean variations ($m\Delta$) between two successive HR determinations in the same child are not significant. Parallel variations of serum IgE are not significant. There is a significant decrease in the clinical score

	Variation of the % HR by anti-IgE				Variation of serum IgE (IU/ml)	Variation of clinical score
	1	10	100	1 000 ng/ml		
$m\Delta$, %	2.2	6.4	9.7	12.5	536	16
$\pm$	3.36	9.08	9.69	14.05	623.40	13.09
Signif.	N.S.	N.S.	N.S.	N.S.	N.S.	$p<0.05$
nb	24	28	28	28	16	24

Table II. *Variation in histamine release in 10 children with AD*

Results of two to three assays at an interval of several weeks. No variations were seen. Low responders remained low responders and high responders remained high responders (see Fig. 1)

	% of histamine release by anti-IgE serum														
	1 ng/ml			10 ng/ml			100 ng/ml			1 000 ng/ml			10 000 ng/ml		
Patient	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Bal.	0	0	—	0	0	—	0	0		0	0.7				
Gay.	0	0	—	0	0	—	0.7	0.7		5	6.3				
Mar.	2	0	—	5	0	3.4	6.1	3.2	4.9	2.5	2	14			3.5
Lar.	0	0		0	0.7		14	16.2		20	14				
Cou.	3	0	0	5	2	3.8	7	2	6.8	26	29	40.6			
Caz.	0	0		0	0		8.5	0.7		56	44				
Fior.	0	0		25.3	43		53.6	55.3		36	31				
Han.	0	1.6	7	40	36	50	82	68	73	79	68	73			
Leg.	7.6	1.9	15.3	15	5.4	20.8	35.5	23.0	21.5	58.9	63.2	63.6			
Pedr.				65.4	70.6		70.6	63.2		16.1	8.0		7.6	3.3	

Table III. *Histamine release in AD and controls*

The percentage of histamine release was significantly higher in children with atopic dermatitis than in controls

	Atopic dermatitis 79.9 $\pm$ 31.5					Controls 56.9 $\pm$ 23.8			
Total histamine content ...									
Anti-IgE, ng/ml ...	1	10	100	1 000	10 000	10	100	1 000	10 000
Histamine release, %									
Mean	0.7	10.2	26.4	32.0*	19.2*	8.7	17.3	12.2*	8.3*
σ	1.6	16.7	24.1	22.2	12.3	14.4	22.0	15.7	10.7
Number of tests	24	29	29	29	5	18	18	18	18

* U: Mann and Whitney, $p<0.01$ for 1 000 and $p<0.05$ for 10 000 ng/ml. Total histamine content was higher in AD than in controls $p<0.01$.

exposure to 1 000 ng/ml anti-IgE (7.16 ± 7.08). Eighteen were found to release more than 25% (47.8 ± 12.05). Total histamine content was similar in both groups (70.5 ± 27 and 85.5 ± 33). No differences in age, age at onset, clinical severity or total serum IgE levels were found between the two subgroups.

DISCUSSION

Two facts emerged from this study. 1) HR by anti-IgE is quite constant in a given child with AD. Such a reproducibility has previously been shown for HR in urticaria (3). There were no great variations between two HR determinations although the clinical state varied dramatically in some children. Therefore, it appears that HR is not correlated with the severity of the disease. 2) On the basis of HR induced by 1 000 ng/ml of anti-IgE it was possible to distinguish two subgroups in children with AD. One had a low response which was quite similar to that of controls. The other had a high response even though the total histamine content was no different from that of the low responder group.

Efforts at subgrouping AD seem important, for instance in the evaluation of the prognosis. We have found that the low responder group did not differ from the high responder group with regard to age, age at onset, clinical severity of the disease, or serum IgE; unlike most of these parameters "releasability" may prove to be an interesting parameter in the evaluation of children with AD. Finally, the theoretical meaning of these findings has to be evaluated, keeping in mind that the technique is performed with whole blood.

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DISCUSSION

Rorsman (Lund). Q: You could in some way relate the difference in the histamine release by your anti-IgE to your IgE level in serum. You need a minimum number of IgE molecules on your basophils to get histamine release. Do you think you did not have enough IgE molecules when you did not obtain histamine release?

A: A little boy who was tested at monthly intervals had a significant variation in serum IgE between 1 000 and 2 000 units, but the releasability was not different. This is a point we cannot explain.

Zachariae (Aarhus). Q: We measured daily urinary excretion of histamine in very severe atopics with high IgE levels and we found only 1/10 with an incrrased excretion. This is completely different from what we found in urticaria and angioedema patients where we found high urinary excretions. I do not want to rule out histamine as a very important agent in atopic dermatitis, but we cannot use it as a parameter.

A: If you look for total histamine in urine you have the 24-hour metabolism. It is impossible that histamine would inhibit chemotaxis, since plasma levels of histamine are so low there. However, in a cell-to-cell contact you may have an increased concentration.

Hanifin (Portland): It has been shown by several workers that the tissue histamine levels are elevated. When we did histamine inhibition of chemotaxis we found very narrow tolerances; molarities of 10^{-4} did not inhibit chemotaxis that 10^{-6} inhibited. So there is a very peculiar relationship there. I think you will have to collect the urine in the middle of the night when they start the flare.

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HISTAMINE RECEPTOR-BEARING MONONUCLEAR CELLS IN ATOPIC DERMATITIS

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Abstract. The number of mononuclear cells bearing membrane receptors for histamine was investigated in peripheral blood from children with atopic dermatitis (AD) by means of the Rosette Histamine Assay, using erythrocytes coated with histamine. Histamine Rosettes (HR) varied from 5.70 to 11.85% in healthy adults; from 3.25 to 7.75% in control children and from 2 to 6.55% in children with AD. Histidine and tryptamine inhibited HR formation slightly, whereas free histamine, histamine H1 and H2 receptor antagonists and the histamine H2 receptor agonist (dimaprit) reduced HR formation much more strongly; no significant difference in inhibition of HR formation by these test agents was found between the three groups of subjects, although the reduction in HR formation by histamine was not constant in children with AD.

Key words: Histamine; Histamine Rosettes (HR); Children with atopic dermatitis (AD)

Over the past few years, numerous workers have shown that monocyte and lymphocyte sub-populations particularly suppressor T cells (7, 8, 12), exhibited membrane receptors for histamine (1, 4, 8). Furthermore, the regulatory roles of histamine via suppressor cells in immune reactions are most probable (5, 7) and significant differences have been observed between atopic and control subjects (10, 12). Using a rosette technique with erythrocytes coated with histamine, we have studied the mononuclear cells (lymphocytes and monocytes) in the peripheral blood of children with AD. Modification of the spontaneous histamine-rosette formation by free histamine, histamine H1 and H2 receptor antagonists and agonists was also studied.

MATERIAL AND METHODS

Technique. The technique used was a slight modification of the method described by Saxon et al. (8). Briefly: peripheral blood mononuclear cells obtained using Ficoll-Hypaque density separation were mixed with sheep red blood cells (SRBC) coated with rabbit-serum-albumin (RSA) or a histamine-rabbit-serum-albumin (H-RSA) conjugate prepared *ad modum* Kedar & Bonavida (3). Then cell suspensions were centrifuged and placed at 0/4°C for 30 min. After staining, (Crystal Violet, 0.5%), cells were examined under the microscope; a rosette was defined as a stained cell which bound three or more erythrocytes. The percentages of HR were expressed as follows: % rosettes (H-RSA − SRBC) − % rosettes (RSA − SRBC).

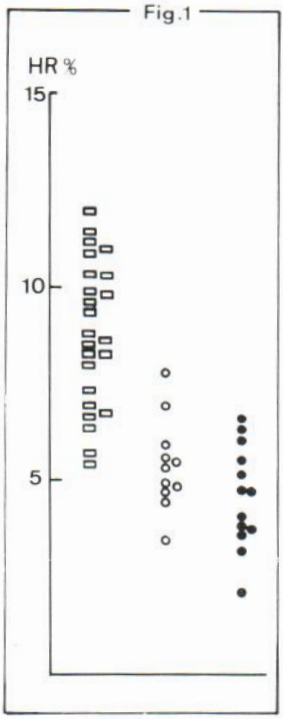
Inhibition of rosette formation was studied as follows: aliquots of the mononuclear cell suspension were incubated with test agents for 30 min. Then the samples were centrifuged, supernatants discarded and cell pellets treated as described above. Test agents were: histidine (100 γ/ml), tryptamine (100 γ/ml) histamine (100 γ/ml), methiamide (50 γ/ml), neoantergan (10 γ/ml) and dimaprit (100 γ/ml).

Subjects. Eight healthy adult volunteers (18/68 years) were studied twice at least, at 1 to 3 months intervals; 24 experiments were performed; 11 non-atopic children (2/15 years) were studied once only; none had lymphoproliferative disease or neoplasia; 11 children (2/14 years) with AD who had not received any treatment for at least one month; 2 were studied twice.

RESULTS

1. Number of mononuclear cells bearing histamine-membrane receptors: Fig. 1 shows that HR levels are significantly lower (U test) in children than in adults ($\alpha < 0.01$). HR are slightly lower in children with AD, but this was not significant as compared with control children. Spontaneous variations in the number of HR were observed: they were slight in most cases in normal adults as well as in 2 children with AD (Table I).

2. Incubations with test agents: Fig. 2 shows that histamine inhibited HR formation significantly more than did histidine and tryptamine (U test, $\alpha < 0.01$). Fig. 2 also shows that there was a significant inhibition of HR formation, not only with histamine, but also with methiamide, neoantergan and dimaprit (Wilcoxon test, $\alpha < 0.01$). The inhibitions induced by histamine and the other test agents were not significantly different in adults and control children as compared with children with AD. However, the inhibition of HR formation by histamine was not constant in children with AD (Fig. 2).



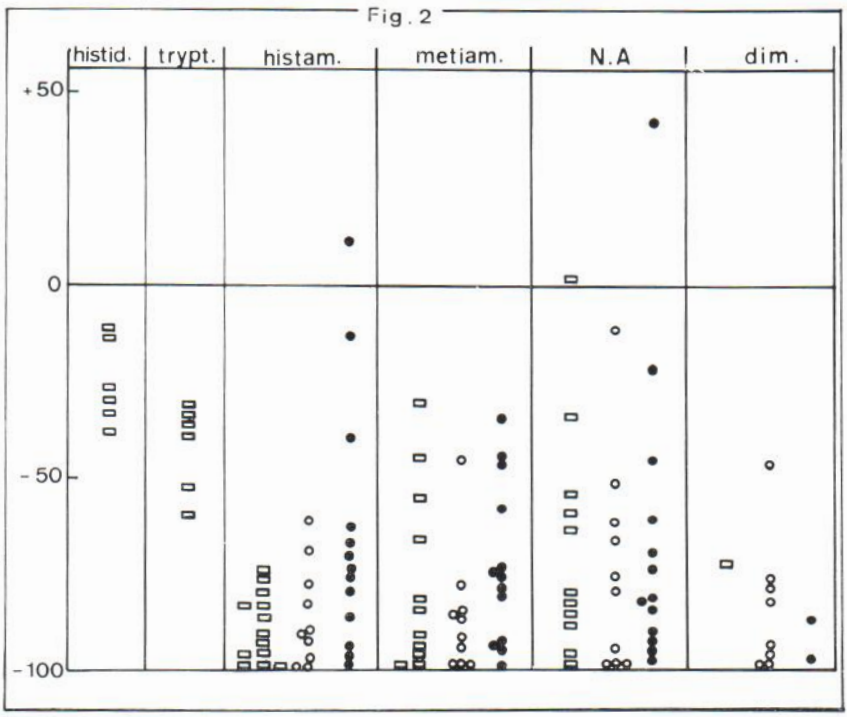


Fig. 1. HR (%) in control adults (□), control children (○) and children with atopic dermatitis (●).

Fig. 2. Inhibition (%) of HR formation by previous incubations with test agents: histidine (Histid.) 100 γ/ml; tryptamine (Trypt.) 100 γ/ml; histamine (Histam.) 100 γ/ml; methiamide (Methaim.) 50 γ/ml; neoantergan (NA) 10 γ/ml, and dimaprit (Dim.) 100 γ/ml, in normal adults (□), control children (○) and children with AD (●). Inhibition (%) was calculated as $[(a-b)/a] \times 100$, where a and b represent HR (%) in preparations preincubated with medium alone or with test agents respectively.

DISCUSSION

The rosette-histamine assay is a highly specific method for the detection of histamine membrane receptor bearing mononuclear cells, in that free histamine, histamine H1 and H2 receptor antagonists and agonists significantly inhibited HR formation, whereas the reduction in HR formation by substances closely related chemically, such as histidine, and other amines (tryptamine) was very slight. Similar results have recently been obtained with guinea-pig macrophages (2); Melmon et al. (4) have previously demonstrated the inhibition by histamine and histamine antagonists of histamine receptor bearing cell adherence to columns of Sepharose beads coated with a H-RSA conjugate.

Table I. *Spontaneous variations in HR (%) formation in healthy adults (cases 1 to 7) and 2 children with AD (cases 8, 9)*

	Tests				
Cases	I	II	III	IV	V
1	8.50	10.70			
2	11.40	5.40			
3	11	8	6.70		
4	6.90	7.40	10.20	8	
5	8.60	9.40	9.60	11.80	6.70
6	10.90	8.30	5.70	8.70	
7	8.30	8.50			
8	6	4.70			
9	5.40	4.80			

The levels of spontaneous HR formation that we have found are lower than those observed by other authors (8). These discrepancies probably result from the choice of control subjects (8) or from technical factors (1). Wide variations in the number of blood mononuclear cells bearing histamine membrane receptors have been observed by these authors; the reasons are so far unclear, but membrane environment (4) and technical considerations seem critical. These factors might also explain the spontaneous individual variations (Table I). In

children, the numbers of histamine rosettes were lower than in adults; this has been suggested previously (1).

The numbers of HR are slightly lower in children with AD than in control children, although the difference is not significant. Suggestive evidence of a suppressor T cell deficiency, which are thought to be histamine-membrane receptor bearing cells, has been obtained in recent studies (11). However, this sub-population represents about 12% of peripheral blood T lymphocytes (8), and decreased numbers of these cells might well not result in decreased numbers of total HR-forming mononuclear cells. Furthermore, this defect seems possibly qualitative as well as quantitative, although the results are sometimes contradictory (6, 9).

In vitro hyperreactivity to histamine has been described in atopic subjects (10, 12). We have found no statistical difference in the inhibition of HR formation by various test agents (histamine, histamine H1 and H2 receptor antagonists and dimaprit) in children with AD as compared with controls. However, in some children with AD, histamine (100 γ/ml) did not inhibit—and even stimulated—HR formation. This was never found in the other groups studied. Preliminary results with dose–response curves suggest an analogous decreased sensitivity of the inhibition of HR formation in children with AD.

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DISCUSSION

Jones (Atlanta). Q: You were unable to competitively block the histamine receptors by addition of histamine in some of the atopic children. How do you interpret that?

A: There are several possible interpretations. One is that the histamine receptors are already saturated in vivo by histamine.

Jones (Atlanta). Q: We have noticed in some of our studies that the histamine receptor may be present at some time in the lifetime of the cell or in a person but not present at other times. Do you have any comments on that?

A: By doing several determinations in the same patients at intervals of several weeks we found variations of something like 30%.

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THYMOSIN-INDUCIBLE 'NULL' CELLS IN ATOPIC CHILDREN

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Thirty children with atopic asthma, thirty with atopic eczema and thirty normal children, all age-matched, were compared. The mean peripheral blood T-lymphocyte level was 1 740/mm³ in the normal group, but significantly lower in the asthma group (mean 970/mm³; $P < 0.0001$) and in the eczema group (mean 1 197/mm³; $P = 0.003$). The T-lymphocyte deficit in both atopic groups was abolished *in vitro* by foetal calf serum or by thymosin, a bovine thymic hormone extract. Analysis of the data from other authors confirms that a T-lymphocyte deficit exists in atopy but is not normally detected when foetal calf serum (or other serum) is used in the E-rosette assay for T-lymphocytes. Positive correlations were found between plasma IgE levels and: severity of atopy, and peripheral blood eosinophil levels. Thymosin-inducible T-lymphocyte (T_i) level correlated strongly with plasma IgE level, suggesting that these T_i-cells may be immature (or blocked) suppressor T-cells. If this T-lymphocyte deficit, whether of a primary or secondary nature, represents inadequate suppression of IgE responses, then a clinical trial of thymosin may be warranted.

Key words: Thymosin; Thymic hormones; T-lymphocytes; Suppressor cells; IgE regulation; Atopy

DISCUSSION

Bonifazi (Bari). Q: We know that total unspecific IgE can persist at hign levels also when the atopic dermatitis has cleared. What about the behaviour of T lymphocytes after the recovery of atopic dermatitis?

A: All our patients were studied in active phase of the disease, so I can't tell you what happens when they go into remission. There is a general trend, but not a hard and fast correlation between T cell levels and IgE levels.

Barnetson (Edinburgh). Q: In our studies we found that patients with eczema alone tended to have normal eosinophil counts and those with respiratory allergy tended to have the highest eosinophil counts. Did you try to divide your patients into those with pure eczema without — and those with respiratory allergy?

A: No, but we will look further at that problem.

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COMMON IMMUNOCHEMISTRY IN ATOPIC DERMATITIS AND BRONCHIAL ASTHMA

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Abstract. Atopic dermatitis and bronchial asthma are closely associated with respect to hereditary factors, epidemiology and occurrence in the same individuals. Both depend on more or less common genetic and environmental traits. Both are typical multifactorial diseases. They have also much in common as regards the complex immunological and chemical mechanisms involved. This becomes particularly evident when comparable subgroups of patients with either atopic dermatitis and bronchial asthma are investigated.

Key words: Atopic dermatitis; Bronchial asthma; *Immediate* hypersensitivity; IgE; Atopy; Immunology; Immunochemistry

Children with infantile atopic dermatitis (AD) run a considerable risk of developing respiratory allergy and bronchial asthma (BA). 50–80% of infants and toddlers with AD have developed BA by 6 years of age (13, 16, 18). In this respect some traits are particularly predictive. In the vast majority of AD infants and children who later develop BA, the respiratory disease is more or less influenced by reaginic allergy (1, 3). Allergy is defined as immunological hypersensitivity which is harmful to the tissues or disruptive of the physiology of the host. There is ample evidence that most reaginic antibodies belong to immunoglobulin E (IgE). Elevated serum and skin IgE and IgE antibodies may be found in a high proportion of children with AD as well as with BA. Particularly high values are found in patients with severe AD, with seasonal allergic asthma and with combinations of AD and allergic asthma or bronchial lability, respectively (7, 15, 18).

The role of IgE-mediated allergy is well documented for several instances of AD and BA (1, 3, 16, 17, 19, 21). However, discussion of the role of IgE immunology in the two diseases can become more than lively on the slightest provocation. When there is so much divergence of opinon, there must be cause for re-evaluation in the light of newer information.

Some of the controversy as regards the role of allergy in AD and BA may be attributed to non-qualified use and non-discriminating interpretations of skin testing and radio-allergosorbent tests (RAST). This applies to both AD and BA. Much of the disagreement may also be due to differences in subpopulations studied. In BA, several subpopulations may be distinguished according to the involvement of immunological and non-immunological factors, respectively (3). A similar distinction may be appropriate even in AD, although the distribution may be quantitatively different in the two diseases.

Comparable subgroups of patients with AD and BA, respectively, have much in common as regards both immunology and biochemistry (Table I).

Itch is the main symptom of AD. Itch may be triggered by many factors, but is a main and prominent feature of allergy. It is the first and most persistent symptom of IgE-mediated reactions in the skin following direct skin testing and passive transfer *a.m.* Prausnitz-Küstner. Itch is also the most frequent skin symptom occurring during inhalant provocation tests in patients with both AD and BA (18). It is also the first—and in some patients, the major—symptom of a local allergic reaction in the nose. For BA, it is possible that in the bronchi the itch reflex is replaced by that of cough..

The wheal and flare reaction as seen in the reversible initial stage of the positive skin test reaction, is set off mainly by histamine released by local mast cells. In the bronchi, allergen challenge results in swelling of the mucosa and bronchial muscular spasm. In the first stage, this is easily reversible in the same way as the initial allergic wheal and flare reaction in the skin. However, in BA as in AD a combination of allergy with special tissue factors seems to be a necessary prerequisite for an acute exacerbation of the disease. Presence of one factor does not exclude the causative

Table I

	Atopic dermatitis		Bronchial asthma	
	Extreme immunol. sub-group	Extreme biochem. sub-group	Extreme immunol. sub-group	Extreme biochem. sub-group
A. *Immunological traits*				
Atopic (IgE) heredity	+	+/−	+	+/−
Elevated skin and serum IgE	+	−	+	−
Serum IgE antibodies	+	−	+	−
Positive skin tests to allergen	+	−	+	−
Positive allergen challenge	+	−	+	−
Non-significant allergies	+	(+)	+	(+)
Incomplete immunological information	+	+	+	+
B. *Biochemical traits*				
Acute and chronic inflammation	+	+	+	+
Abnormal response to histamine	+	+	+	+
Abnormal response to acetylcholine	+	+	+	+
Abnormal response to other biochemical agents	+	+	+	+
Tissue hyper-reactivity	+	+	+	+
Decreased cellular response to adrenergic stimuli	+	+	+	+
Altered autonomic regulation	+	+	+	+
cAMP/cGMP imbalance	+	+	+	+
C. *Clinical traits*				
Occurs in same individual	+	+	+	+
Multifactorial	+	+	+	+
Changeable features	+	+	+	+
Vicious circles	+	+	+	+
Threshold mechanisms	+	+	+	+

role of others. This is amply illustrated for AD by the classical description by Engman et al. (10) of a child with confirmed wheat hypersensitivity. Half his body was protected by dressings and, following ingestion of a wheat cracker, atopic dermatitis lesions appeared, but only on the unprotected areas. This case is consistently referred to, to emphasize the importance of trauma through scratching in the development of AD lesions. It may also, however, be used to emphasize the importance of allergy followed by scratching. If neither is removed in the child, who is usually not bandaged, the child will have eczema.

Neither the pathological anatomy of atopic eczema nor the pathological morphology of BA can be explained by the effects of the immediate and reversible stage of the IgE-mediated reaction as described up to this point (1, 14). However, our concept of the immunochemistry of IgE- and allergen-mediated reaction has been far too naive up to now. Too much consideration has been devoted to the first immediate and reversible stage of biodynamics following allergen + IgE-mediated mast cell release of mediators. The immunochemistry of IgE-mediated reaginic reactions is much more complex. The mast cell acts not only as a transistor and amplifier for the immunological reaction into the immediate and rapidly reversible biochemical reaction as described. It seems to be a leading element also in secondary sub-acute to chronic inflammatory reactions (5, 6). In this respect, it comprises both humoral and cellular aspects of inflammation. In fact, some of the patho-morphological traits found in AD have much in common with the characteristic traits of subgroups of BA (Table I). Many of these traits may be fairly well explained by the tissue reaction set off in the secondary inflammatory stage initiated by mast cell release of biochemically active agents. Clinically, manifestations of the secondary inflammatory stage can be demonstrated both in the skin and in BA (4, 11, 20). The two diseases also have other, possibly related immunological traits in common. Depression of cell-mediated immunity and defective T-cell function as

well as reduced adrenergic responses in lymphocytes are described for both (9, 12, 22).

Neither BA nor AD can be explained by IgE immunology alone. The main non-immunologic characteristic trait in both is hyperirritability and overresponsiveness of the tissues to a number of irritant stimuli. Bronchial hyperreactivity may also be found in patients with uncomplicated AD (15). It is possible that the clinical manifestations depend on genetic regulation of IgE immunology and genetic regulation of particular biochemical tissue reactivities combined (2, 23).

Future research concerned with BA has to concentrate on distinct subpopulations, the one used as control for the other. Data available so far with respect to AD seem to indicate that this should also be the case for this disease. The links between AD, atopic allergy and BA are very strong. This calls for active prophylactic measures to be undertaken in the AD child to prevent or postpone allergic sensitization of the respiratory passages. More should be done to investigate which factors may influence this trend. For a prospective study of etiologic factors in BA, the infant presenting with AD provides the perfect case.

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COMPARATIVE STUDY OF THE IMMUNE RESPONSE INVOLVEMENT IN ATOPIC DERMATITIS AND PSORIASIS

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Abstract. Immunological and pharmacological disturbances in atopic dermatitis and psoriasis are compared. This comparative study revealed several analogies: T cell deficiency; hyperfunction of B cells leading to antibody production, defect in the beta-adrenergic response. The role of immunological and pharmacological factors in the pathogenesis of the two diseases is discussed.

Key words: T cell deficiency; Hyperfunction of B cells; Psoriasis; Atopic dermatitis

Atopic dermatitis (AD) and psoriasis, two major inflammatory skin diseases, differ markedly from a clinical and histopathological point of view. Recently, immunological disturbances have been described in the two diseases and are presumably involved in their pathogenesis. It is surprising to note numerous analogies between AD and psoriasis concerning these immunological factors (8–11). Even more striking is the pharmacological hypothesis indicating a defect of the beta adrenergic response in the two diseases (20–22). In this short paper we try to give a comparative view of the possible immune response involvement in the pathogenesis of AD and psoriasis.

IMMUNOLOGICAL FEATURES

Humoral and immunopathological findings

In AD the most striking feature is the increase in serum IgE level (14). This increase is non-specific (17) and IgG-cytotropic antibodies can be present (19). These IgE could participate in recently found circulating immune complexes (3). IgE-bearing B cells are more common (5). In AD skin, immunoglobulin and complement deposits can be observed (18).

In psoriasis, serum IgA and IgG levels are elevated. High IgE levels are found in 20% of patients, and rheumatoid factors consistent with IgG anti-IgG are present in half of them (6). Circulating immune complexes have recently been described (I–10). Immunoglobulins, complement, rheumatoid factors, antinuclear antibodies and fixed antistratum corneum antibodies are present in involved epidermis and can account for keratinocyte membrane alterations (for review see 8).

In the two diseases these features reflect a hyperactivity of B-cell clones, resulting in a hyperproduction of immunoglobulins.

Cellular immunity

In AD a defect of T cells has been demonstrated in vitro by low levels of E rosettes and low stimulation by PHA and Con A (for review, see 11–9). In our own work (9) the dissociation of decreased E rosettes and normal HTLA values could indicate a defect of T cell maturation, since HTLA appears earlier than sheep red blood cell receptor during the maturation of lymphocytes, but these discrepancies were not found in an other study (2). Moreover, the markedly depressed stimulation by ConA could be in favour of a T-suppressor cell defect.

In psoriasis a similar defect of T cells is demonstrated in the active stages of the disease: a decrease in E and E active rosettes and of HTLA-bearing cells (in opposition to AD), low stimulation by ConA (7). A T-suppressor cell defect has already been suggested (10).

In the two diseases, the impairment of the B-cell control by thymus-dependent lymphocytes could explain the hyperproduction of antibodies.

PHARMACOLOGICAL FEATURES

In AD a defect in the beta-adrenergic receptors has been hypothesized since 1968 (20) but has not been fully demonstrated so far. However, abnormal cAMP responses to beta agonists have been demonstrated in lymphocytes and polymorphonuclear leukocytes which are, on the contrary, normally responsive to prostaglandin E_1 (PGE_1) stimulation (16).

In psoriatic epidermis a decrease in the cAMP/cGMP ratio has been demonstrated since 1972 by Voorhees et al. (22–15) but further studies on epidermal cyclic nucleotides are conflicting (13–24). Adenyl cyclase is poorly responsive to beta agonists but more responsible to PGE2 (23).

In the two diseases, though much work still needs to be done to further clarify this subject, a defect of the beta adrenergic response has been implicated.

PATHOGENIC IMPLICATIONS (Fig. 1)

The possible pathogenic chain of AD could be: defect of T cells → antibody production → mast cell and basophil degranulation → hyperproduction of histamine and other mediators leading to some clinical features of AD (pruritus, oedema, erythema). The beta block could account directly

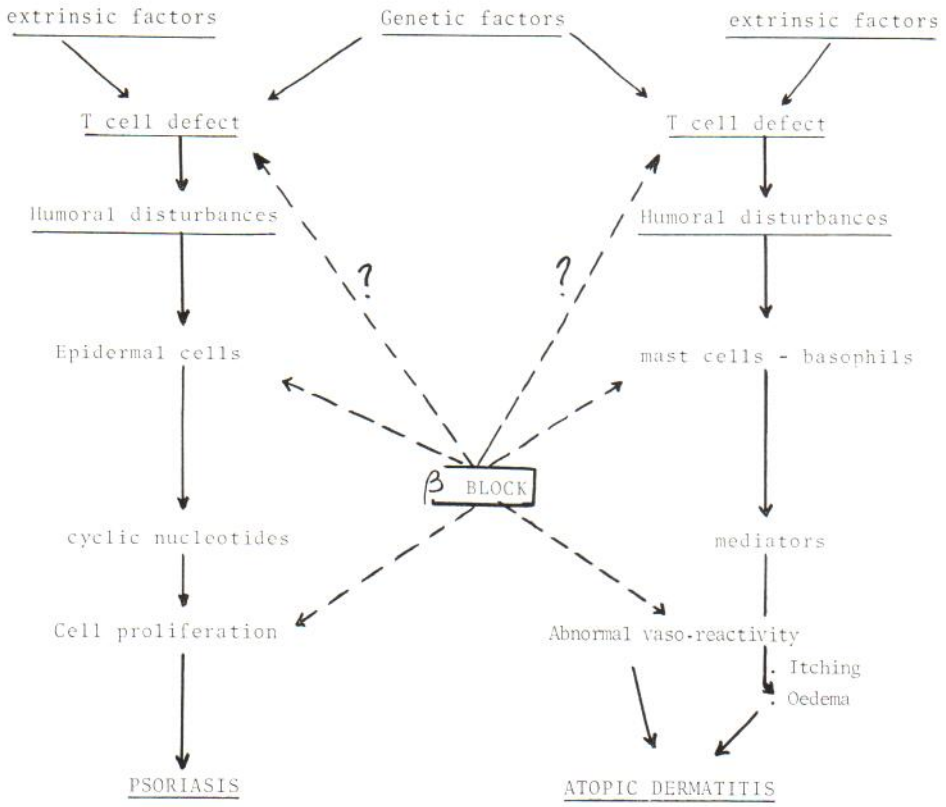

Fig. 1. Hypothetical pathogenic chain of psoriasis and atopic dermatitis and possible role of the β-adrenergic blockade.

for vasomotor and sudoral troubles. It could intervene indirectly via low levels of cAMP in enhancing humoral immunological phenomena and in the imbalance of homeostatic response to mediator secretion, resulting in alpha adrenergic and cholinergic predominant effects.

In psoriasis we have already proposed the following pathogenic chain (10): T cell defect → humoral immunological phenomena, antibodies and complement deposits in epidermis → PMN attaction → enzyme release resulting in keratinocyte membrane alterations. The membrane-bound beta-adrenergic receptor dysfunction could be due to these immunologically induced membrane alterations. This beta-receptor dysfunction could be implicated in epidermal proliferation through cyclic nucleotides and PG_s imbalance.

CONCLUSIONS

In these hypothetical pathogenic chains, some immunological differences leading to different clinical and histopathological aspects are obvious: different humoral disturbances act on different supposed targets, viz. keratinocyte membranes in psoriasis, cells responsible for immediate hypersensitivity in AD. Thus the results differ: epidermal proliferation in psoriasis, effects of hyperproduced mediators in AD.

This comparative study brings out several analogies in the immunological and pharmacological features of AD and psoriasis: (1) T cell defect, (2) hyperfunction of B cells leading to overproduction of antibodies, (3) defect of the beta-adrenergic response. But these analogies might be merely apparent and due to the fact that our knowledge of the two diseases is limited to the basic points only and real immunological and pharmacological mechanisms are poorly understood. For example, we do not know the exact lymphoid subpopulation involved in the T cell defect. This deficient subset may be different in AD and psoriasis. Direct evidence for a T-suppressor cell defect is not yet available and subsets of Fc-γ-bearing T lymphocytes, possibly modulated by immune complexes, could be implicated. The origin of the T cell defect is also under discussion (4). It could be primary, due to genetic predisposition and/or infectious agents; secondary by humoral inhibitors (12), or due to skin inflammation (21).

Likewise, we do not know the role, the origin, the nature or the exact location of the beta-receptor defect; is this deficiency restricted to epidermal cells in psoriasis and more generalized in AD?

Moreover, we are still unable to correlate exactly the immunological data with the pharmacological disturbances. In psoriasis the membrane-bound beta-adrenergic receptor dysfunction could result from epidermal immunological phenomena but inversely in the two diseases immunological features could be due to the abnormal beta-adrenergic response of immune competent cells.

Obviously much more work is necessary to elucidate the exact immunological and pharmacological disturbances in AD and psoriasis and research in immuno-pharmacology is one of the most promising fields for the future.

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TUBERCULIN REACTION IN ATOPIC DERMATITIS

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Abstract. Serial examinations of tuberculin reactivity were performed in 16 patients with atopic dermatitis (AD) and in 14 patients with allergic contact dermatitis. Transient suppression of already established tuberculin reactivity was seen in the contact dermatitis patients. Tuberculin reactivity in the AD patients also fluctuated with the condition of the dermatitis. When the dermatitis was active, there was diminished tuberculin reactivity. During remission of the dermatitis, however, a significant increase in tuberculin reactivity occurred. It is suggested that, in some patients with AD, the suppressed cell-mediated immunity may be secondary to the eczematous inflammatory process.

Key words: Tuberculin reactivity; Cell-mediatied immunity

Patients with atopic dermatitis (AD) often show diminished tuberculin reactivity (2, 4). However, it still remains to be determined whether the hyporeactivity is primary or secondary. I previously reported (5) that, in patients with AD as well as in those with allergic contact dermatitis, their tuberculin reactivity was diminished when the dermatitis was active, and that a significant increase of their tuberculin reactivity occurred while they were in remission or free from the dermatitis.

In the present study, the tuberculin reactivity in each of the patients with atopic dermatitis was examined at the time of exacerbation, during the subsequent remission, and at the re-exacerbation of the dermatitis.

MATERIALS AND METHODS

Patients. A total of 16 patients (age range 16–47 years) suffering from widespread AD, were selected. They all showed seasonal fluctuation in the severity of their dermatitis, which worsened in winter and improved in summer. They received local corticosteroids. Systemic use of corticosteroids was avoided.

Clinical controls consisted of 14 adult patients with widespread allergic contact dermatitis. They too were treated with topical application of corticosteroids.

Skin tests. Each patient was injected intradermally with 0.1 ml of a tuberculin solution (0.5 μg of PPD per 1 ml). The test site was normal-appearing skin on the flexor surface of the forearm. The reaction was read at 48 hours.

To see if a widespread eczematous dermatitis influenced already established tuberculin reactivity, each of the AD patients was tested at the time of the exacerbation of the dermatitis, while the patient was in remission for a period of 4 weeks or more, and when the re-exacerbation of the dermatitis occurred. Patients with allergic contact dermatitis were tested when the dermatitis was active and during a period of 2 to 4 weeks after the disappearance of the dermatitis.

RESULTS

Patients with allergic contact dermatitis. When they had active dermatitis, the mean value of their tuberculin reactions was 3.36 ± 3.49 mm S.D. When tested after the subsidence of the dermatitis, the mean value of their tuberculin reactivity strength was 12.57 ± 6.05 mm S.D. Thus, a widespread allergic contact dermatitis brought about a significant suppression of tuberculin reactivity ($P < 0.01$).

Patients with atopic dermatitis. When they had florid dermatitis, the mean value of their tuberculin reactions was 4.88 ± 5.03 mm S.D. When tested during a period of remission, the mean tuberculin reaction strength was 12.37 ± 10.59 mm S.D. Thus, a significant increase in tuberculin reactivity occurred during the remission of the dermatitis ($P < 0.01$). At the time of re-exacerbation of the dermatitis, the mean value of tuberculin reactions was 5.50 ± 6.12 mm S.D. This was significantly low when compared with the tuberculin reactivity strength during the preceding remission ($P < 0.01$).

DISCUSSION

Transient suppression of tuberculin reactivity was seen in patients with widespread allergic contact dermatitis. Tuberculin reactivity in patients with widespread AD also fluctuated with the condition of the dermatitis. When there was exacerbating AD, they showed diminished tuberculin reactivity. During remission of the dermatitis, however, a significant increase in tuberculin reactivity occurred.

Thus, it is apparent that a widespread eczematous inflammation of varying cause transiently suppresses already established tuberculin reactivity. This association of eczematous inflammation and anergy indicates that, in the evaluation of cell-mediated immunity in patients with atopic dermatitis, it is important to investigate them not only when the dermatitis is active but also while they are in remission.

Several investigators (1, 3) report that the lymphocyte hyporeactivity to PHA often seen in AD patients may normalize during remission of the dermatitis. Therefore, it is possible that, at least in some patients with AD, the diminished cell-mediated immunity may be secondary to the eczematous inflammatory process.

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DISCUSSION

Barnetson (Edinburgh). Q: We have done similar studies and emphatically confirm your observations. We have been studying the lymphocyte transformation responses to *S. aureus* using two different preparations and we have found that during exacerbations of the eczema the responses are greatly diminished and that when the patients go into remission they come up again into the normal range. This does seem to suggest that some sort of non-specific mechanism of anergy comes into play during exacerbations of the eczema and this presumably is something different from the T-cell deficiency which we have all heard described.

Hanifin (Portland). Q: Did you have any patients who were not treated with topical steroids?

A: My patients were treated with topical corticosteroids, but earlier I made guinea pig experiments without using corticosteroids.

Rorsman (Lund). Q: Dr Uehara, do you think there is a vascular background for the phenomenon of the reduced tubeculin reaction? Seeberg & Magnusson found 30 years ago that not only in inflammatory edema but also in cardiovascular edema there was a greatly reduced tuberculin reaction. Furthermore, they observed that if one had an immediate reaction to tuberculin—as one may have sometimes—then one got a very weak response of delayed type because of rapid absorption of the antigen, due to the immediate response.

Wahlberg (Stockholm). Q: Have you considered applying primary irritants at the same time as tuberculin?

A: In an earlier study I used croton oil and formalin as primary irritants. Delayed skin reactions to these substances were quite similar in atopic dermatitis and normal controls.

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AN *IN VITRO* STUDY OF IgE PRODUCTION IN SEVERE ATOPIC DERMATITIS

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Peripheral blood lymphocytes from 4 patients with severe atopic dermatitis and with high serum IgE levels produced measurable amounts of IgE in vitro in repeated tests. These patients had increased numbers of IgE-bearing peripheral blood lymphocytes on at least one test occasion. No measurable IgE production in vitro was found in 6 other patients with atopic dermatitis and in 3 healthy controls. Inhibition of the IgE production was observed following treatment with PHA, Con A, PWM, mixed lymphocyte culture and radiation. LPS and histamine induced neither definite stimulation nor inhibition of IgE production. Supernatants from Con A stimulated cells were used in tests for suppressor factors. The hypothesis that depressed suppressor function of the T cells might be responsible for the tendency to increased IgE production in atopic dermatitis is discussed.

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DISCUSSION

Saurat (Paris). Q: Have you compared the IgE levels in serum with in vitro production of IgE?

A: All the 4 patients who produced IgE in vitro had high IgE levels. The patient who had the highest IgE level in serum also produced most IgE in vitro.

Saurat (Paris). Q: Have you looked at the kinetics of the IgE production, and are you sure that it is a production and not an adsorption and then release?

A: I always counted IgE in the supernatant from cultures where the cells were killed before the culture period and subtracted this when counting production. The amount of IgE increased up to day 7 and after that there was a plateau.

Hanifin (Portland). Q: I was especially interested in the histamine—I think you used T^{-3} Mol histamine to stimulate the cells, and you said there was no stimulation of IgE production?

A: I used histamine in different concentrations but never got anything that could change the IgE production when I introduced histamine from day 0.

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CLASS AND SUBCLASS DISTRIBUTION OF SPECIFIC ANTIBODIES TO CODFISH ALLERGEN IN A PATIENT WITH ATOPIC ALLERGY

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Abstract. Using an indirect ELISA technique together with rabbit antisera specific for human immunoglobulin classes and IgG subclasses, an estimate was made of the contribution of immunoglobulin classes and subclasses to the overall antibody response against codfish allergen in serum from a patient allergic to codfish. Allergen-specific antibodies were found to be of immunoglobulin classes IgM, IgD and IgE and the subclasses IgG1, IgG3, and IgG4. No antibodies of IgA class or IgG2 subclass were present. High levels of IgG3 and IgD were found.

Key words: Atopic allergy; Codfish allergen; Allergen specific antibodies; Immunoglobulin classes and subslcasses

The role of antibodies other than IgE in atopic allergy is a field of growing interest and attention. The interest has concentrated on antibodies belonging to the IgG class, and short-term anaphylactic (11) as well as blocking IgG antibodies have been described (13, 16, 17).

Particular attention has been focused on antibodies of the IgG4 subclass. Elevated serum IgG4 concentrations have been reported in patients with atopic dermatitis (14) and in patients with cystic fibrosis, who have been reported to have a high incidence of immediate-type hypersensitivity reactions (15). A higher incidence of IgG4 was also detected in patients with allergic asthma to castor bean allergen (5), and elevated serum concentrations of IgE and IgG4 were found to correlate with the clinical picture in asthmatic patients (9). Van der Giessen et al. (19) reported that a relatively high proportion of grass pollen specific antibodies belonged to the IgG4 subclass, and an increase in IgE, IgG1 and IgG4 grass pollen-specific antibodies during hyposensitization of hay fever patients has been reported (6).

In the present study we used the indirect enzyme-linked immunosorbent assay (ELISA) to determine the class and subclass distribution of antibodies to the highly purified allergen fraction (DS22) from codfish in serum from a patient allergic to codfish.

MATERIALS AND METHODS

Patient. Serum was obtained from a 22-year-old male with a history of asthma, allergic rhinitis as well as atopic eczema of the severest type with frequent bacterial superinfections. He was allergic to codfish, grass pollen, house dust and animal dander, as determined by a positive history, positive skin prick tests and the presence of specific IgE antibodies measured by Phadebas® RAST kits (Pharmacia, Swedcn). Exposure to codfish allergen induced an attack of asthma. He was treated with topically applied steroid creams and an antihistaminic drug, but no internal steroids. The serum IgE level was raised to 1 540 U/ml (normal: 50–700), serum IgA to 4.4 g/l (normal: 0.5–3.3) and serum IgD to 0.3 g/l (normal: 0.005–0.1), while serum IgG and IgM levels were normal. ESR, haemoglobin and white cell counts were normal.

Antisera. Antiscra were raised in rabbits using conventional immunization procedures. The immunogens used were all highly purified myeloma proteins, some of them purified to homogeneity and characterized by amino acid sequencing.

Immunosorbent techniques. The sera were rendered class or subclass specific by absorption on a series of immunosorbent columns loaded with appropriate myeloma proteins. The myeloma proteins were either coupled to Sepharose 4B (Pharmacia) employing a modification of the CNBr activation method outlined by March et al. (10), or by carboiimide conjugation to AH-Sepharose 4B (Pharmacia). Usually the gels were substituted with protein in a concentration between 1 and 5 mg/ml packed gel. The antiserum against IgG1 was made specific for the Gm markers a, x and f (Glm 1, 2 and 3) by batch absorption with relevant Gm-typed human scra.

Enzyme linked immunosorbent assay (ELISA). The assay was performed in polystyrene tubes (N 1070, NUNC, Denmark) basically as described by Engvall and Perlmann (7). The tubes were coated with the purified codfish allergen fraction DS 22 (usually 100 ng/ml) dissolved in 0.05 M $NaHCO_3$, pH 9.6 with 0.02% NaN_3. After incubation at 4°C overnight, the tubes were emptied by suction and rinsed 3 times in washing buffer consisting of 0.9% saline, 0.02% azide and 0.05% Tween 20 (Sigma Chem. Co., Mo.). If not used immediately, the coated tubes were kept dry and

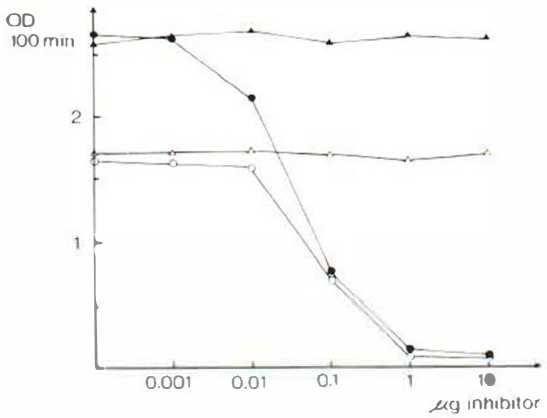

Fig. 1. Specificity test of antisera: Anti-μ antiserum against IgM coat inhibited with an IgM myeloma protein (○) and irrelevant IgG myeloma protein (△). anti-γ3 antiserum against IgG3 coat inhibited with an IgG3 myeloma protein (●) and an IgA myeloma protein (▲).

closed at 4°C. Then the tubes were incubated with a serum sample from the patient (usually 10–20 μl diluted to 1 ml in PBS with NaN_3 and Tween 20, and left for 2 hours at room temperature and 2 hours at 4°C. After washing, the tubes were incubated with saturating amounts of the specific antisera at room temperature. After 4 hours the tubes were emptied and rinsed 3 times with washing buffer before adding 1 ml alkaline phosphatase (ALP) conjugated swine anti-rabbit IgG (Orion Pharmaceutica, Helsinki, Finland) usually diluted 1:500 in PBS with azide and Tween 20. After a further 4 hours' incubation at room temperature the tubes were emptied and washed. Finally, they were filled with 1 ml of enzyme substrate, *p*-nitrophenyl phosphate (PNPP, Sigma Chem. Co., Mo.), 1 mg/ml in 1 M diethanolamine buffer, pH 9.8, containing 1 mM $MgCl_2$ and incubated usually at room temperature, or overnight at 4°C. The enzyme was inactivated by adding 0.1 ml 5 M NaOH and the optical density read at 400 nm. Two sets of blank controls were always included, one in which patient serum were omitted, and one where empty tubes were used instead of coated tubes. The optical density o these blanks was usually well beyond 0.1, and when occasionally it was higher, the whole series was rejected.

Specificity testing of antisera. After absorption the specificity of the antisera was examined by ELISA, employing tubes coated with different myeloma protein (usually 100 ng/ml).

RESULTS

All antisera were shown to be highly specific as they reacted with several myeloma proteins within the same class or subsclass but not across classes or subclasses.

The specificity and strength of the different antisera were further assessed by performing inhibition experiments with the ELISA technique. In these experiments the antisera were preincubated with varying amounts of the inhibiting myeloma proteins in glass tubes bevore being added to the coated polystyrene tubes for further processing as described above. Fig. 1 shows two typical examples of results of specificity tests on antisera. An IgM myeloma protein inhibited the anti-μ antiserum, while an IgG myeloma protein did not. The anti-γ^3 antiserum was inhibited with an IgG_3 myeloma protein, but not with an IgA myeloma protein. Fig. 2 shows the relative amounts of allergen-specific antibodies of various classes and subclasses. Serum from a healthy donor was tested as a reference and no antibodies against codfish were detected. It is seen that in the patient serum, no antibodies of IgA class or IgG_2 subclass were detected. Antibodies of IgG_1, IgG_3 and IgG_4 subclasses and IgM as well as IgD and IgE classes were present and especially the amount of IgD was found to be considerable.

DISCUSSION

The present investigation demonstrates the presence of DS22-specific antibodies of the classes IgM, IgD and IgE, and the subclasses IgG1, IgG3 and IgG4 in serum from a patient allergic to codfish. Antibodies of the IgA class and IgG2 subclass were not detected, although the antisera employed were shown by ELISA to react strongly against relevant myeloma proteins.

The DS22 allergen fraction is a well defined purified allergen fraction from codfish, containing the major allergen M. There are several reports on the identification and characterization of this preparation (1, 2). This allergen was selected because of its protein nature and the purity of the fraction, making coating to polystyrene tubes easy as well as reducing the possibility of non-specific interaction between coat and antisera or conjugate.

The role of the different immunoglobulin classes and subclasses in allergy is a still unsettled issue. While the role of IgE in immediate hypersensitivity reactions seems to be established, the possible role of other immunoglobulins in allergic patients has to be clarified. The IgG4 subclass has been reported to act as a blocking antibody (19, 20) capable of inhibiting reagin-mediated PCA in baboons (17). But a short-term anaphylactic antibody of the IgG class has also been demonstrated (11). Antibodies other than IgG4 and IgE may also be involved and

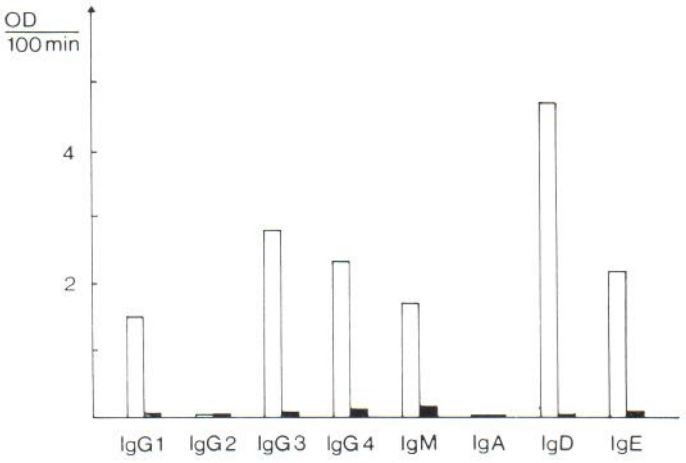

Fig. 2. Reactivity of patient serum (□) and normal serum (■) against DS22-coated tubes as measured by class- and subclass-specific antisera by indirect ELISA.

the presence of allergen-specific antibodies of the other IgG subclasses (i.e. IgG1, IgG2 and IgG3) in allergy have been reported (19). IgA antibodies to allergens have been demonstrated earlier (12). The patient in this study had an increased serum concentration of IgA to 4.4 g/l (normal 0.5–3.3 g/l but in spite of this no allergen-specific antibodies of the IgA class to the codfish allergen could be detected in his serum. This finding may be of special interest in view of the fact that IgA is the predominant immunoglobulin in mucous membranes. The serum concentration of IgD was also elevated, and large quantities of allergen-specific antibodies of the IgD class were detected.

The hypothesis has been presented that atopic individuals with elevated IgE and IgG4 subclass antibodies are likely to have dermatitis (8). Shakib et al. (14) found elevated serum IgE as well as IgG4 in patients with atopic dermatitis, and in the patient material presented by Gwynn et al. (9) 5 children with eczema, asthma and hay fever and 4 adults who had had atopic eczema as children, all had grossly increased concentrations of both IgE and IgG4.

While many patients with atopic dermatitis have very high serum IgE levels, and most patients somewhat elevated levels, some do however have normal IgE values (personal observations). Whether IgG4 serum concentrations are of significance for the presence of atopic dermatitis needs to be more closely investigated.

Bruynzel and Berrens (4) found that the sera of some patients with unequivocal allergies did not contain allergen-specific IgE nor IgG4 antibodies, or else contained solely IgG4 antibodies. Furthermore, in manifest allergy to guinea pig dander, IgG4 antibodies were found more frequently than IgE antibodies. To a lesser extent, the same phenomenon was observed in cat dander and grass pollen allergy.

Antibodies to different antigens may be restricted to IgG subclasses, as has been described for some antigens (19) and Thomas, Watkins & Asherson (18) presented evidence that in mouse, genes in the major histocompatibility complex can selectively control antibody classes. The production of antibodies to antigen is dependent on the nature of the antigen, the mode of exposition, and the immune responsiveness of the individual. Heredity probably plays a major role concerning the observed differences between immune responses of allergic patients and normals.

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DISCUSSION

Aas (Oslo). Q: An inverse correlation between total serum concentration of IgE and IgG when you follow different samples has been described. Have you looked at different samples from your patients with respect to these parameters? A: No, we have only investigated one blood drawing.

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HIGH FREQUENCY OF HEREDITARY COMPLEMENT DEFECTS IN ASSOCIATION WITH ATOPIC DISEASES

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Soothill and Harvey have identified a hereditary immunodeficiency (the yeast opsonization defect) present in the population at genetic polymorphism frequencies, in association with atopy and recurrent infections.

We have studied the complement system in 88 atopic children (including 42 atopic dermatitis cases) by means of several functional tests. Two of these, the "yeast opsonization" test and the "rabbit erythrocyte lysis" test measure two independent mechanisms of activation of the alternative pathway of complement. About 70% of the atopics are defective in at least one of the two tests. Among the atopics who are normal at both tests, some present a functional deficiency of C'2 (and are presumably heterozygotes for a C'2 allele), whereas others present a selective deficiency of immunoglobulins of class A.

Thus, the combined use of a number of immunological tests allows the identification of a biochemical defect (different from the presence of "reaginic antibodies") in the large majority of atopic patients.

DISCUSSION

Strannegård (Göteborg). Q: I understood that there is a faulty functioning of the alternate pathway of complement activation and this must result in a defective killing of virus-infected cells. One would expect atopic individuals to have more persistent virus infections. Does anyone know about frequencies of persistent viral infections in atopic individuals?

A: I have no data on that.

Saurat (Paris). Q: If complement were implicated in atopic dermatitis, would one not expect to have a higher frequency of atopic dermatitis in complement deficiency?

A: A total complement deficiency is one thing, but the alternative pathway complement deficiency is quite another. We do not know a great many things about the latter and diseases. There is no report in the literature about the defect of alternative pathway complement activation and diseases.

Saurat (Paris). Q: What does exactly yeast opsonization measure?

A: This test has been described and is performed as a marker of alternative pathway complement effectors.

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IMMUNOLOGICAL, HISTOLOGICAL AND ELECTRON-MICROSCOPICAL INVESTIGATIONS OF THE GUT IN ATOPIC DERMATITIS

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Abstract. Jejunal biopsy specimens were obtained from ten patients with severe atopic dermatitis and 15 controls. Light microscopical examination of hematoxylin-eosin stained sections showed normal conditions in all atopic patients and intra-epithelial lymphocyte counts did not differ significantly from counts in controls. Scanning electron-microscopical examination demonstrated minor mucosal changes in five of the atopic patients. Immunohistochemical studies did not reveal any significant changes in the class proportions and numbers of jejunal immunoglobulin-producing cells.

Food proteins and intestinal bacterial antigens constitute the major gut-derived antigenic challenge to the body. Recent studies have demonstrated that proteins can penetrate the mature intestinal mucosal barrier under physiological conditions and reach both the submucosa and the systemic circulation as intact, undigested macromolecules (18, 19, 20, 21). Such proteins may function as antigens in the host.

The induction of tolerance is complex and depends among other things on activation of suppressor T cells (2), the filtering role of the liver (17), and the effect of small doses of absorbed antigen which induce a state of "low zone tolerance" (15). One of the most important components of specific host defence is the production of intestinal antibodies; thus secretory IgA plays a major role in the immunological inhibition of absorption of undegraded macromolecular material of food or bacterial origin (1, 10).

Low serum levels of IgA have been reported in healthy infants at the age of 3 months who later became atopic (16), and in children with acute gastroenteritis who later became hypersensitive to cow's milk (9). Furthermore the frequency of IgA deficiency has been reported to be high in patients with atopy (12).

In many cases of atopic dermatitis, allergy to food antigens occurs, and exacerbation of the dermatitis after oral provocation of antigen has been reported (8). In a controlled study, antigen-avoidance diet afforded clinical improvement in children with atopic dermatitis (3), and the beneficial effect of such a diet is known to many clinicians.

To investigate whether patients with atopic dermatitis show intestinal abnormalities, we have performed histological, immunohistochemical and electron-microscopical investigations of the jejunal mucosa obtained from 10 patients with severe atopic dermatitis (AD).

MATERIAL AND METHODS

Patient material. Three females and 7 males, mean age 26.4 years, range 16–38 years, were investigated. All had suffered from dermatitis since early childhood and most of them had a history of allergic rhinitis, asthma or urticaria, in addition to the dermatitis. Mean serum IgE was elevated and all patients demonstrated immediate-type hypersensitivity reactions to various allergens. They received local treatment and antihistamines, but no internal steroids.

Seven men and 8 women, mean age 41 (range 12–77) years served as controls. Most of them had abdominal complaints, but their jejunal mucosa was histologically normal, and extensive investigation did not reveal any organic disease.

Tissue preparations

Several specimens were obtained under fluoroscopic control from the proximal jejunum of each patient by means of a multiple biopsy capsule.

One specimen was fixed in formaldehyde and stained with hematoxylin-eosin for light-microscopical examination. Another specimen was fixed in glutaraldehyde and processed for scanning electron microscopy. A third specimen was fixed in cold 96% ethanol to precipitate diffusible proteins, and the fourth specimen was extracted in cold phosphate-buffered saline for 48 h to facilitate registration of Ig-containing cells (5).

Table I. *Intra-epithelial lymphocyte counts in sections of jejunal mucosa from patients with atopic dermatitis and from controls*

	Number of subjects	Number of lymphocytes per 100 epithelial cells		
		Mean	S.D.	Range
Atopic dermatitis	10	9.5	3.7	5.0–15.6
Controls	10	10.9	2.5	7.7–15.0

Immunohistochemical procedures

Serial paraffin sections were cut at 6 μm and incubated with "green" (fluorescein) and "red" (rhodamine) rabbit IgG conjugates, showing specificity for the five human Ig classes. The characteristics of these reagents, the conjugate combinations used in paired staining, and the fluorescence microscopy conditions have been described previously (5).

Cell registration

Double-exposed colour slides covering a defined "mucosal tissue unit" (4) constituting a 6 μm thick and 500 μm wide block of tissue including the mucosa at full depth from the muscularis mucosae to the surface, were projected on a paper screen. From such registration of paired staining, differential counts of the three major immunocyte classes (IgA-, IgM- and IgG-producing cells) were recorded for each mucosal unit. Data were obtained in absolute figures and as immunocyte class ratios.

Intra-epithelial lymphocytes (6) were counted in hematoxylin-eosin-stained sections and compared with the counts in 10 controls. Data were obtained as number of lymphocytes per 100 epithelial cells; a total of 1 000 epithelial cells were included in each case. Wilcoxon's two-sample test was used for statistical comparisons.

A scanning electron-microscopical study was performed with the glutaraldehyde-fixed specimens.

RESULTS

Examination of hematoxylin-eosin stained sections from the proximal jejunum of 10 patients with severe atopic dermatitis showed normal histological conditions in all patients. Intra-epithelial lymphocyte counts did not differ significantly from the counts in 10 controls (Table I).

Scanning electron-microscopical examination showed some minor changes in 5 of the 10 investigated patients, i.e. occurrence of ridges in a higher number than normal. The height of the villi varied but they were of normal thickness. The surface appeared normal, with characteristic foldings and epithelial pattern.

Immunochistochemical investigation was performed with jejunal specimens from 7 atopic patients and 15 controls. The results are presented in Table II. The mean total number of Ig-producing cells tended to be decreased (but not significantly so) in the atopic patients. No differences were found in the percentage class distribution of the immunocytes. IgD- and IgE-producing cells were rare, but in atopy, cells with a speckled staining for IgE were regularly observed in the lamina propria. These cells were easily distinguished from IgE-producing cells, and apparently represented mucosal mast cells with bound IgE.

DISCUSSION

To our knowledge, this is the first immunohistochemical study of the gut mucosa in atopic dermatitis. A preliminary study of 7 of our atopic patients indicated a reduced number of jejunal Ig-producing cells, although the immunocyte class proportions were found to be unaltered compared with controls. As we felt that some of the mucosal biopsy specimens were of unsatisfactory quality, the study was repeated in a new series of 7 patients (Table 2); in only one case did the total number of Ig-producing cells fall below the normal lower range (52 vs. 86 cells), and the atopic group as a whole showed only a slight trend towards reduced cell number. Moreover, the immunocyte class proportions were again quite normal. This study, therefore, gives no good support for the idea that a defect of the secretory IgA system may give rise to an increased absorption of intestinal antigens as part of the pathogenesis of AD. However, it does not preclude that such a defect may be present temporarily during the sensibilization period, and individual variations may well persist. The possibility of a qualitative defect in the secretory IgA system should also be con-

Table II. *Distribution of immunoglobulin-producing cells in an average "mucosal tissue unit" of jejunal mucosa from patients with atopic dermatitis and from controls*

	AD patients ($n=7$)	Controls ($n=15$)
Mean percentages (observed range)		
IgA	80 (64–89)	79 (67–88)
IgM	17 (10–25)	18 (10–31)
IgG	3.0 (0.3–14)	2.6 (0.6–6)
Number of cells (Observed range)	122 (52–210)	131 (86–199)

sidered. The presence of IgE-bearing mast cells in the gut mucosa indicates that type I hypersentitivity reactions may occur locally and contribute towards increased intestinal permeability; this observation is consistent with that of Feltkamp-Vroom et al. (11) in other tissues of atopic patients.

In 5 of 10 patients, scanning electron-microscopical examination revealed some minor morphological changes, i.e. occurrence of ridges in a higher number than normal. In our preliminary series, minor changes were found in 4 of 7 patients. The significance of this observation is not clear. The light microscopical examination showed normal conditions, and the intra-epithelial lymphocyte counts did not differ from the control counts.

Matuchansky et al. (14) found in 5 of 10 cases with constitutional eczema a parietal villous atrophy localized most frequently to the proximal small intestine, without surface enterocyte anomalies. Conversely, Fry et al. (7) did not find any significant abnormality of the gross appearance of the mucosa in 4 cases of atopic eczema, and Marks & Shuster (13) concluded that the frequency distribution of both predominant and individual small intestinal mucosal features in patients with eczema differs very little from a local control population, in agreement with our observations.

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DISCUSSION

Barnetson (Edinburgh). Q: Might I suggest in future studies that you try to provoke changes in the small intestine by giving the patient an allergen?

A: That is of course a possibility but these patients were in-patients with a severe dermatitis, so you might say that they were already provoked.

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THE ATOPIC-CHRONIC-DERMATOPHYTOSIS SYNDROME

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Abstract. This article reviews the clinical and laboratory aspects of the relationship between atopy and dermatophytosis. This newly appreciated relationship; chronic, stabilized dermatophytosis and bronchial asthma and/or allergic rhinitis, constitutes a clinical syndrome of importance and interest. The relevant host defense; correlate, cell mediated immunity, is subject to the modulating effects of the mediators of Immunoglobulin-E mediated hypersensitivity in the atopic. Evidence suggests that IgE, the mast cell and histamine act locally within the loose connective tissue of the skin to inhibit T effector cell function and inflammation. The final common pathway for histamine as an immune modulator is unknown but may involve binding to H-2 receptors on the plasma membrane of lymphocytes or endothelial cells. The consequent reduction in intensity of inflammation within the lesion permits establishment of a chronic stabilized dermatophyte infection. Recognition of the atopic-chronic-dermatophytosis syndrome draws attention to the underlying immune mechanism which may have broader implications in biology.

Key words: Atopy; Dermatophyte; Immunoglobulin E; Mast cell; Trichophytin; Immune modulation; Histamine

Because atopy and fungal infection are each common, one would expect occasional coexistence of these diseases in the same patient. In recent years, a relationship has been noted between atopy and chronic, extensive dermatophyte infection which appears to be more than coincidental (5, 7–10, 14).

First, Lobitz et al. (14) described a severe atopic dermatitis patient who had generalized *Trichophyton rubrum* infection. Second, Jones et al. (7), in a 1973 study of dermatophytosis in prison inmates observed that 40% of the inmates who harbored chronic and extensive dermatophyte infection were atopic. Their atopic manifestations were almost exclusively hay fever and/or asthma. Chronic dermatophytosis was 3 times more frequent in those inmates having atopic manifestations.

Subsequently, Hanifin et al. (5) reported that approximately 50% of 49 patients with chronic *Trichophyton rubrum* infection had a personal or family history of atopy. Sorensen & Jones (21) found 9 of 17 chronic dermatophytosis patients to have a personal or family history of hay fever, asthma, or atopic dermatitis. These studies establish a relationship between dermatophytosis and atopy which suggests that atopy is associated with a predisposition to acquire chronic infection (see Table I).

Factors which predispose to dermatophytosis. To place the relationship between dermatophytosis and atopy in proper perspective it is necessary to review what is known to affect an individual's susceptibility to dermatophyte infection (see Table II). Early case reports suggested that individuals with lymphoma and Cushing's disease were more susceptible to dermatophytosis (13, 15). Immunosuppression from systemic medications and topical steroids may be observed to heighten susceptibility to fungal infection including dermatophytosis (12), although how common this occurs is not well documented. In addition, some believe diabetes mellitus predisposes to dermatophyte infection (6), although convincing evidence is lacking.

A new syndrome. Thus atopy, chiefly mild hay fever and/or asthma appears to be the only identifiable condition consistently and frequently associated with chronic and extensive dermatophytosis. To draw attention to the association between these two conditions perhaps we should denote the relationship as the atopic-chronic-dermatophytosis syndrome.

Clinical and laboratory characteristics of atopic individuals who have chronic and extensive dermatophytosis

Clinical characteristics. Typically, the atopic-chronic-dermatophytosis patient is a male who contracts his initial infection in the second decade of life. The personal atopic history typically consists of childhood asthma and/or hay fever; in many the respiratory problems will have resolved spontaneously before dermatophytosis begins. At the time of evaluation, *Trichophyton rubrum* is commonly found to be the infecting fungus. Most patients claim that the

Table I. *Reports which establish relationship between atopy and chronic dermatophytosis*

Authors	Year	No. with chronic infection	No. atopic
Lobitz et al.	1972	1	1
Jones et al.	1973	34	14
Hanifin et al.[a]	1974	49[a]	24[a]
Sorensen/Jones	1976	17	9
Totals		101	48

[a] Hanifin et al. stated that approximately 50% were atopic.

initial manifestation of infection was an intensely inflammed lesion, but with time although never actually resolving, the lesion became less intensely inflammed and spread. The infection will frequently have been present for more than 20 years.

The chronically infected lesion is characterized by pruritus, erythema, scaling and hyperkeratosis. Inflammation is not intense, in fact is minimal. Typically the feet are involved in an erythrodermic, hyperkeratotic moccasin-type pattern. The palmar and plantar skin may develop painful fissures. The crural region, buttocks, thighs, finger and toe nails are commonly affected. When the condition is generalized the picture may be that of an exfoliative erythroderma.

Laboratory findings. The significant laboratory findings are related to the immune system (see Table III). There is enhanced IgE synthesis and decreased cellular immunity. The finding of normal levels of immunoglobulins G, M and A are contrasted with a mild or moderate elevation of serum IgE which again reminds one of the relationship to atopy. In keeping with their personal and family history of atopy, the atopic-chronic-dermatophytosis patient has multiple positive immediate scratch and intradermal tests to common environmental allergens. Intradermal injection of trichophytin, a soluble dermatophyte glycopeptide, typically elicits a large immediate wheal and flare reaction. RAST studies have confirmed that serum from such individuals contains IgE reactive with trichophytin (R. D. King et al., unpublished data).

Table II. *Conditions which may herald a predisposition to chronic, extensive dermatophytosis*

Condition	Frequency found
1. Atopy, especially hay fever and asthma	Commonly
2. Diabetes mellitus	? ? ?
3. Immunosuppression due to medications	Less commonly
4. Lymphoma/thymoma	Uncommonly
5. Cushing's syndrome	Rarely
6. Primary immunodeficiency diseases	Rarely

Table III. *Anticipated immunological findings in the atopic-chronic-dermatophytosis syndrome*

1. Mild-moderate increase serum IgE
2. Multiple positive scratch and intradermal tests to common environmental allergens
3. Immediate hypersensitivity reaction to trichophytin antigen
4. Trichophytin specific IgE by RAST
5. Weak or absent delayed hypersensitivity to trichophytin
6. Weak or absent *in vitro* lymphocyte response to trichophytin
7. Otherwise normal immune function

In contrast to the presence of IgE reactive to trichophytin, cell-mediated immunity to trichophytin is decreased. Intradermal injection of trichophytin typically reveals weak or absent delayed-type hypersensitivity (DTH) to that antigen (10). Lobitz et al. (14) found their atopic-chronic-dermatophytosis patient who had absent DTH to trichophytin to have a severe, general depression of cellular immune reactivity which was reflected both *in vivo* and *in vitro*. It should be noted that this patient had severe atopic dermatitis. Hanifin et al. (5) and Sorensen & Jones (21) could not confirm the presence of a general depression of CMI function in their atopic-chronic-dermatophytosis patients, most of whom had mild respiratory atopy. In fact their patients reacted normally to a battery of delayed-type hypersensitivity skin tests with the exception of one antigen, trichophytin. Hanifin et al. (5) used lymphocyte transformation to confirm *in vitro* the decreased cellular immune response to trichophytin. These reports suggest that the respiratory atopic with chronic dermatophytosis has a minimal and possible antigen specific decrease in cellular immunity. In atopic dermatitis patients the defect in cellular immunity may be more severe.

Characteristic pattern of immune reactivity to trichophytin. Thus the immune responsiveness of the atopic-chronic-dermatophytosis patient is characterized by: (1) enhanced synthesis of IgE to trichophytin and several other antigens, and (2) a relative specific failure to express cell-mediated immunity to trichophytin both *in vivo* and *in vitro*. This distinctive pattern of immune response to trichophytin is intriguing.

Basis of the atopics susceptibility to dermatophytosis

An immunologic predisposition. The immunologic predisposition of the atopic may play an important role in the host parasite struggle, leading to weakened host defenses and chronic dermatophytosis.

Background. Before addressing the question of which host defense mechanism fails in the atopic-

Table IV. *Host defense value of the various immune responses to dermatophyte infection*

Response	Value
Immunoglobulin	
G	Not defined
M	Not defined
E	Negative
Cell mediated immunity	Positive

chronic-dermatophytosis patient we must first review the immune response to these fungi and evaluate the role of each response in host defense (see Table IV). The principle antigens involved are complex glycopeptides referred to as trichophytin. The frequency and titer of IgG and IgM antibody are low. The IgE response to trichophytin is stronger and more frequent. Investigators have not been able to show that any class of antibody plays a positive role in host defense against dermatophytes. In fact, the occurrence and highest titer of antitrichophytin antibody, especially IgE, is most closely correlated with severe infection.

In contrast, studies of cellular immunity to dermatophytes, both in animals and humans, have shown a positive correlation with intact host defense. Also the expected corollary, that compromised cellular immunity to trichophytin would be associated with susceptibility to infection, has been documented. Just how intact cellular immunity to trichophytin provides host defense against dermatophyte fungi is not known. In this regard it may be important that dermatophyte fungi survive poorly in intensely inflammed skin.

It is not widely known that the cellular immune response to trichophytin produces essentially all the inflammation at a dermatophyte infected site (9). The fungal parasite, even though proliferating rapidly, has little capacity to incite intense inflammation. When, however, an immunized host expresses cell-mediated immunity to dermatophyte antigen at the site of infection the involved skin becomes acutely and intensely inflammed. Development of intense inflammation heralds the disappearance of the dermatophyte and subsequent resolution of the infection.

Furthermore, acquisition of cell-mediated immunity to trichophytin via a primary infection is associated with relative resistance to reinfection (9). The exact mechanism mediating host resistance to dermatophytes has not been determined. It has been suggested that intense inflammation may impeed fungal invasion by damaging the epidermis and permitting inhibitory plasma factors access to the pathogen. Inflammation also accelerates epidermal turnover (shedding) (2). In any event, intact cell-mediated immunity to trichophytin and the intense cutaneous inflammation produced within infected skin appears to be the bulwark of host defense against dermatophytes.

How is host defense compromised in the atopic? Now, with the necessary background we may address the question of which host defense mechanism fails in the atopic-chronic-dermatophytosis patient. Atopic patients with chronic dermatophyte infection frequently claim that for a short time their infection was intensely inflammed. Since the host CMI response, and not the parasite, determines the degree of inflammation the change in intensity of inflammation suggests something has altered the host immunity or the inflammation it produces. Experimental dermatophyte infection in an atopic with a past history of asthma and hay fever permitted Jones et al. (10) to document the decrease in inflammation and study the changes occurring in the immune-inflammatory mechanism. The change from an intensely to a minimally inflammed infection occurred 4 weeks into the subjects primary infection and was associated with a dramatic emergence of immediate hypersensitivity to trichophytin. Beginning at that point in the host-parasite interaction delayed-type hypersensitivity to trichophytin was demonstrated to decrease and most importantly the infected lesions became less inflammatory. Spread by continguous extension as well as development of new areas followed. These observations of natural and experimental dermatophyte infection strongly suggest that the synthesis of IgE to dermatophyte antigen is central to the defect in host resistance which predisposes to the atopic-chronic-dermatophytosis syndrome.

The role of IgE in immune modulation of T effector cells

Jones et al. (7–11) suggested that IgE was critically involved via the mast cell and its complement of pharmacoactive mediators in modulation of T-effector cell function in chronic dermatophytosis. They hypothesized that trichophytin, which is water soluble, diffuses into the dermis from the fungus

which parasitizes only the stratum corneum. In the dermis trichophytin would bind specifically to sensitized T-cells or specific antibody. Thus mast cell fixed antitrichophytin IgE would become a trigger that when pulled by trichophytin would activate mast cell secretion of pharmacologic mediators. In this scheme locally released histamine would act on antigen activated T-effector cells within the area temporarily prohibiting further activation and release of lymphokine or other mediators of inflammation. Thus, histamine would inhibit T-effector cell function and suppress delayed-type hypersentitivity mediated inflammation.

Jones et al. (10) presented evidence to support this hypothesis. First, Prausnitz-Küstner (PK) transfer of trichophytin-specific IgE into the skin of a subject exhibiting only trichophytin-delayed hypersensitivity (DH) produced, with antigen challenge a typical immediate hypersensitivity reaction followed in 48/72 hours by a 50% reduction in DH. In a similar manner, local intracutaneous injection of histamine or agents causing release of histamine produced a muting of the delayed hypersensitivity reaction. This strongly suggests that histamine can suppress the delayed hypersensitivity reaction to trichophytin.

Subsequently, evidence accumulating from several laboratories has suggested that histamine exerts this effect via specific receptors (H-2 receptors) located on the T-effector cell membrane (1, 3, 16, 18). The changes in T-effector cell function are thought implemented internally through adenyl cyclase and intracellular cyclic nucleotide regulation of cell function. More recent evidence suggests that histamine may be an intermediate and not the final mediator in this pathway. Rocklin (19) has reported that histamine causes lymphocytes to release a protein of approximately 23,000 to 42,000 molecular weight referred to as Histamine Suppressor Factor (HSF) which inhibits sensitized lymphocytes from proliferating and producing MIF. Furthermore, Schwartz et al. (20) have reported that in mice, serotonin may modulate cutaneous delayed hypersensitivity (DH). Schwartz's group proposed that serotonin does not act directly on the T-cell but on cells of the vascular endothelium, altering the permeability of specialized venules to bone marrow derived mononuclear cells critical to the cutaneous DH reaction.

Plausability of IgE-mast cell-mediator modulation theory. Whichever final common pathway is operative remains to be clarified. Nevertheless, it seems that the IgE-mast cell mediator system is an important mechanism for in-situ immune modulation. The IgE-mast cell mediator modulation theory completely explains several puzzling findings characteristic of the atopic-chronic-dermatophytosis syndrome namely (1) basis of the atopics susceptibility to dermatophyte infection; (2) decrease in inflammation concomitant with appearance of trichophytin specific IgE and (3) antigen specific nature of CMI defect.

An alternative sequence of events capable of leading to chronic dermaphytosis

It is not known if all atopics who contract chronic dermatophytosis do so through the sequence of events discussed above. It is theoretically possible for the atopic to acquire an identical immunological profile (enhanced IgE and depressed CMI to trichophytin) plus a chronic infection through an entirely different process.

The alternative scenario which requires years to be fully expressed is based on immunization through the respiratory passages by non-pathogenic but antigenetically cross reactive fungi. This results in an IgE response that later in life thwarts CMI to antigen from pathogenic dermatophyte fungi which penetrates through the skin. This is a plausible alternative supported by several findings. First, the non-pathogenic molds have been shown to contain antigens which are cross reactive with trichophytin (17). Second, some individuals with asthma and hay fever synthesize IgE very early in life in response to inhalation of airborne molds or fungi. Such individuals when examined at 10–12 years of age don't express CMI to trichophytin or antigens of the airborne molds, yet they exhibit strong immediate type hypersensitivity to airborne mold antigens and to trichophytin (8). Thus, although never infected with dermatophytes these children exhibit the immune profile of the atopic-chronic-dermatophytosis syndrome patient. Should the skin of such an individual subsequently become infected with a dermatophyte what would transpire is unknown. It would seem that even if CMI to trichophytin developed the presence of cross reactive IgE would, via the aforementioned modulation mechanism, inhibit T-effector cells. Thus, chronic dermatophytosis could develop without the individual having ever expressed CMI to the infecting organism.

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DISCUSSION

Rorsman (Lund). Q: I would like to have your opinion on the possibility that the decreased delayed reactions to trichophytin that you observed could be due to a change of vascular reaction only. Could it be due to the edema you have created by your immediate response so that the antigen was transported away?

A: I don't think one can exclude that at all. There is, however, some data from intracutaneous tuberculin testing with a radio-labelled tuberculin that leads one to believe that a fair amount of the antigen remains at the sites.

Strannegård (Göteborg). Q: Is the primary thing that T lymphocytes are more sensitive to histamine in these cases than in normal individuals, or would you imply that there is a modulation course of increased IgE levels?

A: I don't know. Maybe both.

Vickers (Liverpool). Q: I found cases with fungal infections between the toes but widespread fungal infections are rare in Britain.

A: I have never observed a patient with atopic dermatitis who also had an extensive dermatophyte infection as well.

Saurat (Paris). We have seen patients coming from North Africa, many with numerous atopic features, and having granulomas in the scalp.

Zachariae (Aarhus). A: We have a group of about 50 patients who have the same immunological pattern as described by Jones. We have noted the same laboratory findings. Almost all of these patients have an atopic disposition but none have atopic dermatitis. They usually have the clinical picture of trichophyton rubrum infection affecting one hand and a sole, now and then the soles alone.

Aas (Oslo): The reactions to trichophytin depend very much on the preparation, which may vary from batch to batch. Some mould extracts, for example, contain both high and low molecular irritant and endotoxins, so that one can have no allergic irritant reactions in the skin without significance. So it is very important to have defined materials and standardized methods.

A: I agree with that completely. The glycopeptide which we used was prepared by ourselves over a 3-year period and was irritant-free and toxin-free.

Saurat (Paris). Q: When you give patients with trichophytoses griseofulvin for a long time, they improve. Have you observed any modification in the immune responses?

A: If one's therapy is effective and the infection is eliminated, the cellular immune response may come back. In some it will, in some it won't.

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ON THE SIGNIFICANCE OF THE TRICHOPHYTIN REACTIVITY IN ATOPIC DERMATITIS

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Patients with pure atopic dermatitis with and without tinea infection were investigated and compared with patients with long-lasting tinea infections and with controls, for presence of intracutaneous reactivity to trichophytin, Penicillium, Cladosporium and Alternaria antigens. RAST for Penicillium and Cladosporium was also performed. The results showed a lack of delayed reactivity, but an immediate reactivity to trichophytin in 50%/40% of atopic patients with/without tinea infection. Non-atopics infected with tinea showed 66% immediate and 33% delayed response to trichophytin. The reactivities in atopic dermatitis (but not in the non-atopic group) were general parallel with mould reactions tested intracutaneously or by RAST. It is assumed that a positive trichophytin reaction in atopic dermatitis does not necessarily mean sensitization to dermatophytes, but is primarily the sign of a cross sensitivity to moulds.

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DISCUSSION

Jones (Atlanta): I agree that the IgE antibody to airborne molds and not sensitization to trichophytin may be the primary route, but we saw no detectable reactivity until the 32nd day in experimental trichophytin infection.

Q: Do your patients with persistent fungal infections develop later, in other conditions such as lymphomas?

A: During the time I observed, they did not, but this was not long enough to answer the question.

Vickers (Liverpool): I have only rarely seen in England severe atopic dermatitis patients with widespread fungal infections. Does this depend on genetically different material?

Jones: I have never observed an atopic dermatitis patient with widespread extensive fungal infection. My patients had atopic respiratory disease and mild eczema.

A: That was an interesting point. In spite of some climatologic resemblances to Great Britain, our material is different. I will clarify that I have not seen extensive fungal infections in my patients, but long-lasting, therapy-resistant ones.

Hanifin (Portland): The overall incidence of fungal infection is decreased in atopic dermatitis, perhaps due to shedding of the skin unfavourable for dermatophytes. Perhaps we have cases where the atopic dermatitis obscures the fungal infection and they are thus not detected. So, in rare instances, atopic dermatitis patients do develop very striking dermatophytosis.

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RESULTS OF FOOD TESTING IN ATOPIC DERMATITIS

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Abstract. Skin testing to find food allergy in atopic dermatitis is recommended from the age of 4 months up to early adulthood. Scratch, scratch-chamber, prick and intracutaneous tests may be used. In order to obtain optimal benefit from skin testing, both commercial extracts and fresh foodstuffs must be used. Extracts of protein-rich foods, such as fish, egg and nuts, work very well in skin tests, but fruits and vegetablas lose their allergenicity easily in the extraction process and that is why they are tested in raw state. The younger the patient the better the correlation between the skin test results and the challenge tests. The nature of the allergen also has a great influence on the clinical relevancy of the skin tests. Extracts of various cereals correlate for example in almost every case with skin reactivity alone and not with symptoms from ingested cereals.

Key words: Skin testing: Atopic dermatitis; Food allergy; Immediate hypersensitivity

In the thirties and forties food testing was popular in many countries. In the fifties and sixties it played only a minor part in routine testing for detecting allergens responsible for flare-ups in atopic dermatitis. This decade has seen the revival of skin tests, antigen-avoidance diets, peroral challenge tests and hypoallergenic food regimens in the diagnosis and management of atopic disorders. Laboratory tests have been developed for detecting allergens, to purify them and to detect specific IgE antibodies in the sera of the allergic patients. Some dermatologists and pediatricians use skin tests as a screen for food allergies, while others rely neither on skin tests nor on RAST-test results.

In this paper I shall discuss our observations concerning the usefulness of scratch, prick and intracutaneous tests and the clinical relevancy of the test results.

SYMPTOMS AND SIGNS OF FOOD ALLERGY (Table I)

Widespread dermatitis with sudden flare-ups, eczema on the eyelids and perioral area and oedema in the lips, oral mucosa and eyelids are clues to food hypersensitivity. Allergic rhinitis, asthma, conjunctivitis and gastrointestinal disorders may also accompany other signs and sometimes they are the only symptoms of this type of allergy.

Contact urticaria from foodstuffs is nearly always due to immediate hypersensitivity, and in most cases local symptoms in mouth and nasopharynx and/or aches and pains in the stomach are noticed (5).

ALLERGENS SUITABLE FOR SKIN TESTING (Table II)

Kjell Aas (1, 2) has studied cod fish allergy and been able to demonstrate the proteins responsible for the reaction. In fact, the results of skin tests with fish extracts as well as extracts from egg, peanut and other nuts often agree closely with the patient histories and peroral challenge tests (7). On the other hand, several fruits, vegetables and edible roots lose their allergenicity very easily in cooking, deep-freezing and juice-making (5, 9). Spices can also be tested as such, but extracts for prick and intracutaneous testing can also be made. Meat, liver and other protein-rich foods are suitable for scratch testing, and commercial extracts are also available. Allergy to meat, however, is so rate that these allergens are needed in sporadic cases only.

The difficulties in producing a proper test substance for skin testing are very pronounced in cereals. A positive skin test usually means nothing more than cutaneous allergy (10). In such cases, cereals do not elicit any signs of hypersensitivity when the patient eats bread or porridge. We rarely find exceptions to this rule. Young children may show a positive correlation between the skin test result and peroral challenge in rice and corn hypersensitivity. In buckwheat allergy a skin test is also relevant in adult patients. Extracts

Table I. *Symptoms and signs suggesting food allergy in atopic dermatitis*

Symptoms and signs	Foods usually responsible
Periorbital and perioral dermatitis	Fruits and vegetables (apples, carrots, etc.). Spices
Sudden aggravation of the dermatitis elsewhere	Egg, milk, cereals
Itching and edema on lips, tongue and in throat with or without rhinitis and conjunctivitis	Fruits, vegetables, peas, soybean
Urticarial rashes	Plum, spices

Table II. *Food testing in atopic dermatitis*

SC = scratch chamber test, P = prick test, I = intracutaneous test, S = scratch test

Allergen(s)		Skin test method	Relevancy	Remarks
Fish	Commercial extract	P I	Excellent	One of the most important allergens throughout life
Egg	Commercial extract As such	P I S	Good	Hypersensitivity gradually wanes
Milk	Commercial extract As such	P I S	Good in babies	Hypersensitivity usually disappears between 1 and 2 years
Nuts	Commercial extract	P I	Good	Cross allergy to birch pollen in 90–100%
Peas Soybean	Commercial extract As such (ground)	P I S	Good in babies	Hypersensitivity gradually wanes
Spices	As such Extracts	S SC P I	Variable	False-positive skin test reactions occur
Carrot Swede	As such As such	S SC	Good	False-positive and false-negative reactions occur. Cross allergy to birch pollen
Plum Pear Apple Peach	As such	S SC	Good	Group allergy between the fruits. Cross allergy to birch pollen
Celery	As sucb Extract	S SC P	Good	Severe test reactions may occur. Does not lose its allergenicity by cooking. Cross allergy to birch pollen
Potato	As such	S SC	Good	Loses its allergenicity by cooking
Paprika	Extract As such	P I S	Moderate	False-positive reactions occur
Meat	As such	S SC	Poor	False-negative reactions
Liver Kidney	As such As such	S SC	Poor	A positive reaction suggests cutaneous sensitivity
Cereals	As such (flour) Extracts	S P I	Poor in adults moderate in babies	Positive reactions in cutaneous allergy only in adults
Buckwheat	As such Extract	S P I	Good	Large test reactions may occur

from cereals may be used for prick and intracutaneous tests, but flours in scratch tests as such also elicit positive reactions nearly as often as the extracts.

METHODS FOR SKIN TESTING

Prick and intracutaneous tests are suitable for most extracts which have been sterilized and which are pyrogen-free. A scratch test is the method used for most of the fresh vegetables and fruits, for natural spices and flours as well as for meat. The scratch-chamber test can also be used especially in testing apple, carrot, potato and other allergens that are easily destroyed (5, 6). In this test a small amount of crushed test substance is placed in an ordinary epicutaneous test chamber (Finn Chamber®, Epitest Ltd Oy, Helsinki) and fixed on the skin of the patient's back for 15–20 minutes. Then the test material is removed and the results recorded. A positive response is a wheal and flare reaction, at least half the size of the reaction produced by histamine 10 mg/ml.

Allergic contact urticaria is sometimes also seen on intact skin and more easily on diseased skin (8). This type of testing is indicated, however, especially when looking for causes of type I contact eczema, although a positive result is a clue to allergic symptoms from ingested foods as well (11, 12).

PATIENT'S AGE

Usually it has been said that only children of 4 and upwards are old enough for skin testing with foods. In to our experience, younger children can also be tested. Babies from 4 to 12 months of age are calm and do not become angry and cry when small scratches are made on their backs. It seems that at this age the test results are clinically highly relevant and irritant reactions are very seldom seen (Table III). Among the most common allergens only tomato, rye, barley and mustard can produce non-specific irritan

Table III. *Food allergy in atopic dermatitis. Results of skin testing*

Foodstuffs	Patients							
	4–12 months 7 pats		>12–36 months 21 pats		Over 36 months 54 pats		Total 82 pats	
	++ +++ (relevant)[a] No.	+ No.	++ +++ No.	+ No.	++ +++ No.	+ No.	++ +++ No.	+ No.
Milk (Bencard)	2 (2)		3 (1)		3 (0)	2 (0)	8 (3)	2 (0)
Egg (Bencard)	3 (3)		2 (2)		1 (1)	2 (0)	6 (6)	2 (0)
Fish (Bencard)	1 (1)		3 (2)	1 (1)	4 (2)		8 (5)	1 (1)
Spices as such			2 (2)		9 (4)	10 (0)	11 (6)	10 (0)
Cereals as such								
Rice as such		1 (1)			1 (1)			
Rice as such			1 (1)				1 (1)	
Corn as such								
Pea as such		1 (0)	1 (0)		7 (2)		8 (2)	1 (0)
Soy bean as such					4 (1)		4 (1)	
Potato as such			1 (0)		7 (3)	1 (0)	8 (3)	1 (0)
Carrot as such		1 (0)		1 (0)	10 (5)	3 (1)	10 (5)	5 (1)
Apple as such	1 (0)				11 (8)	1 (0)	12 (8)	1 (0)
Tomato as such					7 (1)	3 (0)	7 (1)	3 (0)
Negative to all	3 pats		8 pats		24 pats		35 pats	
False-negative results to	banana		egg, fish, wheat, pea		Apple 2, wheat 2			

[a] Patient history or challenge test positive.

reactions. Children between 1 and 4 years of age may be so worried about testing that it is impossible to perform any kind of skin test on them. At that age the correlation between skin test results and challenge tests declines.

Foods causing allergic symptoms in older children and young adults differ greatly from those in younger children. In babies, milk and egg allergies are common. Milk allergy usually disappears during the second year of life and hypersensitivity to egg diminishes to a great extent at 10–20 years. New allergens become important: apple, carrot, swede and various spices. On the other hand, allergy to pea, soybean and cereals may remain unchanged for years. Persons allergic to birch pollen are often hypersensitive to fruits and vegetables (3, 5). True cross allergy between birch pollen and apple has been demonstrated (4), and such a cross allergy obviously exists also between birch pollen and potato and carrot.

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DISCUSSION

Hanifin (Portland). Q: The majority of my patients have facial involvement, but they are not all sensitive to foods. What are the subtle features that allowed you to distinguish and be suspicious of food allergy?

A: About 1/3 to 1/2 of patients with periorbital and perioral eczema are allergic to foods.

Atherton (London). Q: I must confess that I am not too convinced that there are any clinical signs which may enable us to distinguish between patients allergic to food or not.

Aoki (Osaka). Q: Do you make your extracts yourself or are they commercial extracts? We do not see many positive reactions in our department.

A: We use commercial extract of fish, milk and eggs and that is all.

Aas (Oslo). Q: When you say "relevant", do you mean in giving the patient eczematous flares when they eat it, or do you mean relevant as an allergen?

A: With relevant reactions I mean that the patient gets eczema—or sometimes urticaria (usually eczema).

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HISTORY OF FOOD ALLERGY, RAST AND CHALLENGE TEST IN ATOPIC DERMATITIS

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Abstract. In 541 patients with atopic dermatitis (AD), a history with special reference to food allergy, RAST and challenge tests were performed. In 84 patients cutaneous symptoms (exacerbation of the dermatitis or acute urticaria) were attributed to ingestion of various foods, especially eggs, milk, fish and peaches. While in cases of acute urticaria the history agreed largely with the results of the RAST and challenge test, in patients with exacerbation of the dermatitis, considerable discrepancies came to light between the anamnestic data and the other tests. Therefore the essential role of food allergy, in a consistent number of cases of atopic dermatitis, is unlikely. In the subjects with a family history of atopy, the early introduction of artificial milk neither increases the incidence of atopic dermatitis, nor precipitates its earlier onset, nor—finally—increases the incidence of asthma or food allergy. Only 3 out of 25 subjects with specific IgE antibodies to nuts showed specific IgE to birch, which is an uncommon tree in the South of Italy.

Key words: Dermatitis; Atopic; Food allergy; RAST

Atopic dermatitis (AD) is a most complex condition in whose pathogenesis, which has not yet been fully elucidated, genetic, environmental and immunological factors play an important role.

The aim of this paper is to elucidate the etiopathogenetic significance of food allergy in AD, with special reference to the type of feeding in early infancy.

MATERIAL AND METHODS

541 subjects with AD who came under our care in the last 5 years were admitted to the study. A majority of the subjects, namely 503 (248 males), were under 12 years of age and 38 (11 males) were 13 to 44 years of age. In addition our series included 80 controls aged 1 to 12 years and 12 controls aged 13 to 50 years, all suffering from other diseases and with neither personal nor family history of atopy.

In all these subjects a detailed allergological history was obtained with special reference to the possible cutaneous manifestations of food allergy. Furthermore, in 6 000 children who came under our care in the last 18 months, a special inquiry was undertaken into the family history (parents, children, brothers) of atopy (AD, asthma, rhinitis) and into the type of feeding in the first 3 months of life.

Specific IgE antibodies to white of egg, milk, and fish (cod) were measured by radioimmunoassay, using the kit Phadebas-RAST (Pharmacia, Uppsala). In a further 84 subjects antibodies were measured to nuts and almonds. In 25 subjects with antibodies to nuts, RAST was carried out with three allergens. The results were expressed on an arbitrary scale from 0 (negative) to 4 (1, 2, 3, 4 positive) as previously described (3).

Table I. *Clinical history: cutaneous symptoms elicited by the most allergenic foods*

	Number of patients with	
	Urticaria	Eczematous lesions
Egg	15	24
Milk	2	14
Peaches	14	2
Fish	12	2

In all these subjects challenge tests were carried out. In those subjects already put on a diet excluding certain foodstuffs, challenge tests were carried out under double-blind conditions. Others were first put on a diet free from the offending foodstuff or foodstuffs for 30 days and then challenge tests were carried out, starting with very small doses and increasing the latter progressively by repeated administration over a period of at least one week. In cases of doubtful results, the tests were repeated in various seasons, sometimes using the double-blind technique.

RESULTS

History. Among the 541 subjects with AD, cutaneous symptoms after ingestion of eggs, milk, peaches and

Table II. *Atopic diseases in 222 subjects with family history of atopy*

	Breastfed (150)	Bottlefed (72)
Atopic dermatitis (AD)	88 59%[a]	23 32%[a]
Onset of AD (mean age)	18 months	20 months
AD + asthma	24 27%[b]	4 17%[b]
AD + food allergy	22 25%[b]	3 13%[b]

[a] Of the total cases. [b] Of the cases with AD.

Table III. *Results of RAST and clinical history (cases with RAST positive/total cases)*

	Food allergy		No food allergy
	Urticaria	Eczem. lesions	
Egg	14/15	18/24 (2)	135/502 (76)
Milk	2/2	8/14	96/525 (57)
Fish	11/12	1/2	32/528 (14)

( ) = RAST class 1

fish were reported in 85 cases. Other foods were responsible for cutaneous symptoms in 24 cases. Details of symptoms associated with the most offending foods are shown in Table I. No instance of food allergy was recorded in 92 non-atopic patients.

Inquiry into the type of feeding in infancy allowed us to single out, among the subjects at risk —222 patients with a family history of atopy— 2 quite distinct populations: 1) babies exclusively breastfed for at least 3 months; 2) those on bottle feeding from day 1. The results are listed in Table II.

RAST. Results of RAST with egg, milk and fish are presented in Table III. In 3/25 subjects with antibodies to nuts, antibodies to birch were also demonstrated. In 15 of these 25 cases antibodies to juniper (in 4 patients), to oak (in 13 patients), and to plane-trees (in 12 patients) were also shown, (Table IV).

Table IV. *Antibodies to trees in 15 subjects with antibodies to nuts (figures indicate RAST class)*

	Betula	Juniperus	Quercus	Olea	Platanus
1. C. M.	2	1	2	0	2
2. I. A.	0	0	0	0	0
3. D. S.	0	0	3	2	0
4. O. M.	0	1	2	1	2
5. P. A.	0	0	0	0	0
6. L. A.	0	0	2	0	2
7. Z. R.	1	2	2	2	2
8. F. M.	0	0	2	2	2
9. D. G.	0	0	2	0	1
10. C. G.	0	0	2	1	2
11. M. D.	0	0	2	0	2
12. R. M.	2	0	2	2	2
13. V. M.	0	1	2	3	2
14. M. A.	0	0	1	0	2
15. N. G.	0		2	0	2

Table V. *Egg sensitivity in atopic dermatitis (AD): Clinical data, RAST and challenge test (CT)*

	RAST class				
	0	1	2	3	4
In 15 cases of AD + egg urticaria	1		9 (6)	1 (1)	4 (4)
In 24 cases of AD + egg exacerbation	6	2 -1-	7 -2-	3 -2-	6 -3-
In 502 cases of AD without fiood allergy	367	76	48 -2-	10 -3-	1
In 92 controls without AD	90	2			

( ) = number of patients with CT positive: urticaria. - - = number of patients with CT positive: exacerbation AD.

Challenge test. In patients reporting a history of acute manifestations (urticaria, angioedema, rash) the results of challenge tests proved positive in 34/43 cases. In patients with a history of exacerbation of the dermatitis, elimination diets and challenge tests were positive in 13/42 cases. In patients with positive RAST and negative clinical history, elimination diets and challenge tests were positive in 5/253 cases.

DISCUSSION

Introduction of heterogeneous proteins in the first months of life may, in some cases, render a latent atopic constitution more evident. However, in a majority of our subjects at risk of atopy, early introduction of artificial cow's milk neither increased the incidence of AD, nor precipitated its earlier onset, nor—finally—increased the incidence of asthma and food allergy. On the contrary the data presented in Table II indicate a surprising prevalence of atopic disorders in breastfed babies. These data are in agreement with those of Halpern (5), who followed for 7 years 1 753 children fed breast milk, soy milk, or cow's milk: the development of allergy was similar in all three groups.

Recently Björksten & Saarinen (2) were able to detect cow's milk antibodies only in breast-fed children. They suggested that large quantities of antigen, such as in children fed cow's milk, may inhibit the IgE response.

The foods reported more frequently as allergenic in our series were eggs, milk, fish and peaches. The cutaneous response developing after ingestion of eggs was either acute (urticaria) or eczematous in

type. That recorded after ingestion of milk was usually of the latter variety and that after fish and peaches consisted mainly of acute manifestations.

The most common acute pattern was an attack of urticaria, usually of the "contact type". The onset was immediate after contact with a raw white of egg, with fish and with the skin of peaches.

The picture of eczematous type took longer to develop and the time lag was more variable (6–72 hours).

The age was of considerable importance. Cutaneous reactions occurred mostly in the first 10 years of life, only to become attenuated and often disappear altogether later, while AD, might still persist.

Acute reactions of food allergy were more frequently demonstrated in our patients with AD and respiratory allergy. Some authors (1,4) pointed out the association between respiratory allergy and food sensitivity. One possible explanation was cross-sensitivity between birch pollen and hazel-nut allergen, but in our series, antibodies to nuts were more frequently associated with antibodies to oak and plane-trees. By contrast, antibodies to birch and juniper, which are rare trees in our country, were seldom demonstrated.

In our previous paper (3) the role of the most common food allergens in the etiopathogenesis of AD was investigated. Antibodies to foods can be frequently demonstrated in AD patients, but they are not always responsible for exacerbation of dermatitis. This is especially evident in the case of antibodies to eggs. The data presented in Table IV show that these antibodies can be found in 1/3 of AD cases (167/541), but they were responsible for eczematous lesions in only 13 cases (2.4%), confirmed by double-blind challenge tests. It would appear therefore that food allergy may constitute a factor responsible for eczematous lesions in some cases of AD, but its essential role in a significant group of cases is unlikely.

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DISCUSSION

Larsen (Aarhus). Q: In the cow's milk group and the breast-fed group, how many patients in the two groups had a double parental history of atopy?

A: We did not make this differentiation.

Rajka (Oslo). Q: Have you seen itch as the first sign in your challenge experiment?

A: Yes, itch is a very important symptom which we find in acute reactions such as urticaria and in eczematous flares and exacerbations of the dermatitis. In the acute reaction pruritus begins a few minutes after challenge. In eczematous lesions, pruritus began between 6 and 72 hours after challenge.

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HYPERIMMUNOGLOBULINAEMIA E IN ATOPIC ECZEMA (ATOPIC DERMATITIS) IS ASSOCIATED WITH "FOOD ALLERGY"

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Abstract. Thirty-two adult patients with atopic eczema were compared with a similar group of atopics with asthma and/or rhinitis. Twelve patients with eczema had a history of food allergy, either to fish or eggs: only one of the asthma/rhinitis group gave such a history. When "prick" testing to foods was performed, all but one of these patients with a history of food allergy had a positive skin test to foods. In addition, 6 further patients with eczema and one further patient from the asthma/rhinitis group gave positive skin tests to foods. Total serum IgE concentrations were estimated in each patient, and it was found that those patients with the highest IgE concentrations were those with positive skin tests to foods. As severe atopic eczema in adults is a difficult management problem, it is suggested that in those with positive skin tests to foods, exclusion diets are worth a trial.

Key words: Immunoglobulin E; Atopic eczema; Food allergy

Patients with atopic eczema tend to have very high IgE concentrations (1–3), and this is particularly true if the eczema is associated with asthma and rhinitis (4). A majority of authors (3, 5–6), have also asserted that there is a positive relationship between the severity of the eczema and the total serum IgE concentrations though it is generally accepted there is considerable variation between the serum IgE concentrations in different patients, and that the concentrations in individuals are relatively constant when sequential studies are performed.

The present study was designed to ascertain the reason for the variation in serum IgE concentrations between different patients with atopic eczema.

PATIENTS AND METHODS

Thirty-two patients with atopic eczema were studied. Eighteen were male and 14 female, and their ages ranged from 18 to 54 years; 10 of them also had a history of asthma and rhinitis, 8 had asthma, and 3 hayfever. They were compared with 28 patients with asthma and/or allergic rhinitis who were matched for age and sex (9 had a history of asthma and rhinitis, 6 had asthma, and 9 had rhinitis), and 28 matched healthy controls.

The patients from each group, and the healthy controls had venous blood withdrawn for total serum IgE concentrations to be estimated: this estimation was performed using the solid-phase radio-immune-adsorbent technique (Pharmacia, Uppsala, Sweden). Each patient and control was also skin-tested by "prick-testing" to 8 inhalants and 8 food allergens: the allergens were supplied by Bencard, Brentford, Middlesex, and the following were used: (*a*) inhalants: pollens from mixed grasses, pollens from mixed flowers and shrubs, dog hair, cat fur, horse hair, house dust, house dust mite and Aspergillus fumigatus; (*b*) foods: mixed cereals, vegetables, meats, fruits, fish, shellfish, cow's milk and whole egg. The results of skin tests were recorded after 15 minutes and were deemed to be positive when the weal reaction had a diameter (on average) greater than 5 mm; lesser weals were disregarded. Statistical significance for differences in serum IgE concentrations between groups was calculated in each case using the Mann Whitney U test.

RESULTS

History of food allergy

Patients with eczema. Of the 32 patients with eczema, 11 gave a history of allergy to foods with swelling of the mouth and throat, and in some, vomiting. Of these, 6 gave a history of allergy to fish, 4 to eggs, and 2 to both allergens. (One other patient noted that her eczema deteriorated after ingestion of fish but had no symptoms of angio-oedema). Only one patient had a history of allergy to milk, and he had since "outgrown" it.

Patients with asthma and allergic rhinitis. Of the 28 patients, only one gave a history of food allergy, having experienced angio-oedema on ingestion of fish.

Control group. Not one of the 28 healthy controls had a history of food allergy.

Skin testing

Patients with eczema. Of the 32 patients, 29 had one or more positive prick tests to inhalants, and 17 had one or more positive prick tests to foods (Fig. 1). In all but one of the patients with a history of

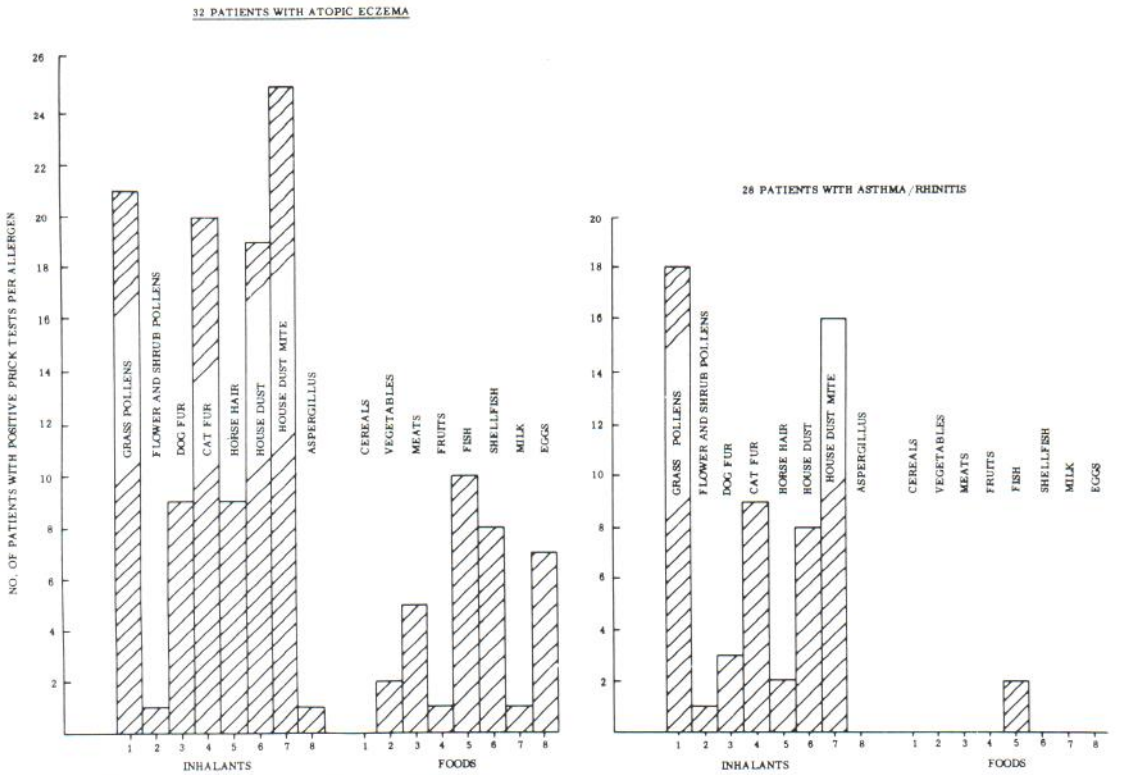

Fig. 1. Number of patients with positive prick tests per allergen (inhalants and foods): 32 patients with atopic eczema and 28 patients with asthma and/or rhinitis.

allergy to foods, this was confirmed by a positive prick test with weal >5 mm diameter (in the one patient with a history of food allergy whose prick test did not achieve a weal of 5 mm diameter, the weal had a diameter of 4 mm). In addition, these patients and 6 further patients produced unexpected positive skin tests to foods, both to fish and eggs, and also to shellfish, and meats (on subsequent testing, mainly to pork). Only one had a positive prick test to milk, and there was no history of allergy to milk in this patient.

Patients with asthma and/or rhinitis. Twenty-four of the 28 patients had positive prick tests to inhalants. Only two had a positive prick test to foods (fish), one being the patient who gave a history of allergy to fish (Fig. 1).

Control group. Two of the 28 controls had unexpected positive skin tests to grass pollens.

Total serum IgE concentrations

The total serum IgE concentrations of patients with atopic eczema (median 4 000 U/ml) were significantly higher ($p < 0.01$) than those of patients with asthma and/or rhinitis (median 300 U/ml) and the control group (median 45 U/ml). However, there was noted to be a marked variation in IgE concentrations in the eczema group (range 10–40 000 U/ml).

When the serum IgE concentrations in all groups of patients with atopy were correlated with the results of skin tests to food allergens, it was found that those patients with positive prick tests to foods (median 9 500 U/ml) had significantly higher IgE concentrations ($p < 0.01$) than those without. It was also observed that the eczema patients without positive skin tests to foods, had similar IgE concentrations (median 450 U/ml) to those of the asthma/rhinitis group (median 280 U/ml); and the IgE concentrations were again significantly lower ($p < 0.01$) than those of the skin test positive (to foods) group (Fig. 2).

DISCUSSION

These studies show that the IgE concentrations are highest in those atopics with evidence of "food allergy", and suggest that antibodies to food are

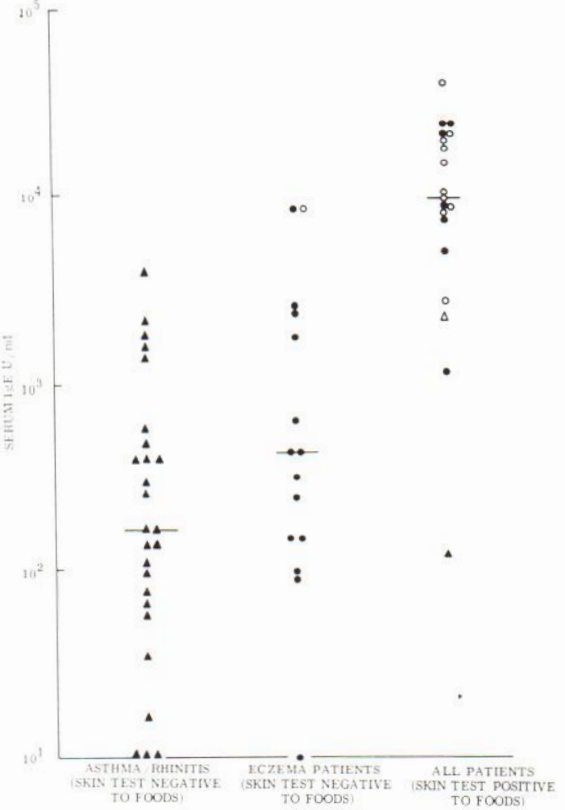

Fig. 2. Serum IgE concentrations in patients with positive skin tests to foods compared to those with negative skin tests to foods. Open circles (○) or triangles (△) indicate those with history of angio-oedema following ingestion of foods.

largely responsible for the very high IgE concentrations seen in atopic eczema patients.

Patients with severe atopic eczema who tend to have high IgE concentrations, are a difficult management problem. Patients with mild atopic eczema are usually effectively controlled by the application of steroid ointments, the use of antibacterials where necessary, and the avoidance (as far as possible) of precipitating factors such as stress, excessive sweating and contact with irritants such as wool: however, on the whole, these measures are not adequate to control those with severe eczema. If food allergy (latent or overt) plays even a minor part in the pathogenesis of the eczema component, then avoidance of potential allergens could be helpful.

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DISCUSSION

Aoki (Osaka). Q: Is there any difference between patients who are simply allergic to food and others who are also sensitive to moulds in the effect of food restrictions?

A: I have not studied that. My main message is that the higher the IgE, the more foods they are allergic to.

Aas (Oslo). Q: I would like to comment on heredity. Links between genetic markers and allergens have only been demonstrated with rare and less active allergens.

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TREATMENT OF ATOPIC DERMATITIS WITH TOLEROGENIC DOSES OF ANTIGEN

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Abstract. The principles of allergic management of atopic dermatitis, with special reference to sublingual hyposensitization, are briefly elucidated.

Key words: Tolerance; Sublingual hyposensitization

Atopic dermatitis from an allergist's point of view is simply one form of allergy. The physiology of atopic skin is analogous to the physiology of asthmatic lungs. Many scientific studies have clearly shown that there is a condition of beta-adrenergic blockade with an imbalance of cyclic AMP vs. cyclic GMP in both skin and lungs. There is also probably a T-cell defect in atopic dermatitis and asthma, with decreased T-suppressor cells and increased production of IgE antibodies. The genetic defect which allows these conditions to occur may cause atopic dermatitis, nasal allergy, asthma, and any of the whole spectrum of allergic diseases.

The life-time variation of atopic disease is quite clear. The initial symptoms of atopy in the infant are frequently atopic dermatitis involving the cheeks, which usually clears during the second year of life. The nasal symptoms may occur at the same time but are frequently delayed into childhood. If the severity of the problem is great enough, with a family history of wheezing, then asthma may occur. Infection, heavy antigen exposures, and stress factors, account for some of the changes in the cycle.

Hyposensitization has not been particularly successful in atopic dermatitis and is not clearly recommended in its treatment. The conventional use of increasing dosages of injectable antigens to produce blocking antibody has not given good results in atopic dermatitis.

The only allergic condition clearly benefiting from blocking antibodies has been stinging insect allergy. This is a special type of allergy where a small amount of antigen is injected by the insect into the subject. In this type of exposure it has clearly been shown that blocking antibodies can be passively transferred and are effective in preventing the allergic reaction.

The end result of successful long-term allergic management is the development of tolerance, with decreased IgE and IgG antibodies. This would be an example of high dose tolerance. The immunologic development of low dose tolerance in animals has had little clinical use in man. Low dose tolerance can be achieved in man by the frequent administration of small doses of antigen which produce decreased IgE antibodies and evidence of immune tolerance. I have utilized the technique over the last 12 years in treating various allergic disorders.

The primary allergic management of atopic dermatitis must include a broad approach to the allergy problem. Inhalants tested and treated must include dust, dust mite, pollens, epidermals, and molds. Various techniques must be used to carefully diagnose food intolerance and chemical susceptibility. It has been shown that part of the problem in certain patients with atopic dermatitis who have a marked elevation of IgE, have excessive IgE antibodies specific to the bacteria which releases histamine and interferes with the body's immune response. Bacterial antigens that cause skin infections should be included in the diagnosis and treatment of atopic dermatitis. To make matters more complicated, certain materials such as formaldehyde must be considered as both contact and inhalant problems. The inhalation of formaldehyde fumes from new fabrics, carpeting, foam insulation, and wall board, constitute definite hazards which are probably more of an allergic nature than simple toxicity.

Careful evaluation of the patient with atopic dermatitis for inhalants can be done by intradermal titration and radioallergosorbent (RAST) for allergens. Elimination diets followed by sublingual provocative food testing and subsequent feeding challenges can be used for foods. Radioallergo-

sorbent (RAST) tests can be of some help for foods. Chemicals can be tested by intradermal and provocative techniques. Inhalants and chemicals are treated with proper sublingual dosage of antigen three times daily under the tongue. Foods are also treated sublingually before meals and as needed with food antigen. Response of patients under treatment can be judged by their clinical response, by their decreased skin reactivity, and by improvement in the radioallergosorbent tests. The dosage of inhalant antigen is increased as the patient's sensitivity decreases.

This treatment lends itself to the early treatment of atopic problems. After 25 years of medical practice, it is clear to me that the problem infant, if properly recognized and diagnosed as atopic, can be very effectively treated, and that the natural development of further allergic problems is retarded or completely reversed. Atopic dermatitis is an allergic disease which can be treated by using tolerogenic doses of antigens.

DISCUSSION

Brandrup (Copenhagen). Q: Can you argue for your statement that cracking of the chin and fissures by the ear lobe are connected with mold/house dust allergy?

A: I do have to rely on my experience, but I think my observations are correct.

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DIETARY ANTIGEN AVOIDANCE IN THE TREATMENT OF ATOPIC DERMATITIS

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Abstract. There is now good evidence that food allergy is an important aetiological factor in atopic dermatitis and that dietary antigen avoidance is a helpful form of therapy, particularly in younger children. Allergy history, prick tests and the RAST are of limited value in identifying the allergies present in individual children. A systematic practical approach to allergy diagnosis is currently under evaluation.

Key words: Dermatitis; Atopic; Food sensitivity; RAST

The role of food allergy in the aetiology of atopic dermatitis has received little attention from dermatologists, paediatricians and immunologists alike in recent years. Relatively few children give a clear history of exacerbation of their dermatitis by foods. Although prick tests to food antigens are frequently positive, avoidance of the test food frequently produces no noticeable improvement. For these reasons, antigen exclusion diets have for some time been unfashionable in the treatment of atopic dermatitis. The conviction that benefit results from the avoidance of certain foods, especially cows' milk and eggs, is nevertheless a common phenomenon among the parents of children with atopic dermatitis. Dietary treatment has been advocated by allergists, particularly in Scandinavia and in the USA, though little objective evidence of its therapeutic value has appeared in the literature. The finding, that exclusive breast feeding can reduce the incidence of atopic dermatitis in predisposed infants (2), certainly adds support to the concept that this disease might be a consequence of sensitization to food antigens occurring during early life. However, the mechanism by which such an effect is achieved has not been established, and avoidance of cows' milk protein per se may not be the most important factor.

There was a clear need for a properly controlled study of the effect of dietary antigen avoidance in atopic dermatitis. In designing such a study we decided to concentrate attention on children under 9 years, though we excluded those below 2 years for ethical reasons. We selected, empirically, a diet excluding eggs and cows' milk primarily, but also chicken and beef because these share some common proteins. A major problem was to create an appropriate control regime against which to test the antigen avoidance diet. Rigid exclusion of certain foods in a diet administered in the child's home requires the full co-operation of the parents and cannot be achieved without their full understanding. The maintenance of 'blind' conditions was overcome by the use of a 'sham' diet. Eggs, cows' milk, chicken and beef were avoided in *both* the 'trial' diet and the control diet. A milk 'substitute' was given during both periods, consisting of a dried soya preparation during the 'trial' diet, and a mixture of dried cows' milk and egg during the control diet. Although these milk substitutes tasted different, both had a flavour unfamiliar to the patients and their parents; they were not informed of the nature of the milk substitutes. We invited 36 children to take part in the 12 week study; all had typical atopic dermatitis and at least one positive prick test to a standard battery. Each diet was given for a 4-week period with an intervening 4-week period when the children resumed their usual diet. The order of allocation of the diets was randomised and unknown to the dermatologists making the clinical assessments. The results of this trial have been published in detail (1). Significantly greater clinical improvement was observed during the trial diet period than during the control period. 12 out of the 20 children completing the study experienced really worthwhile benefit from antigen avoidance; we have follow-up data for 11 of these. Seven of these 11 still find dietary antigen avoidance helpful 2 years later. All have tried reintroducing the excluded foods. Reintroduction of eggs led to exacerbation of eczema in all of these 7 children who continue to be on diets, and cows' milk reintroduction caused exacerbations in 6 of these 7. The majority can

	Prick tests to milk and egg antigens	
	+	-
Response to antigen avoidance	6	6
No response to antigen avoidance	5	2

Fig. 1. Numbers of patients having positive prick tests (weal >2 mm) to at least one of 5 egg and cows' milk preparations before dietary egg and cows' milk avoidance, according to subsequent clinical response.

now eat beef and chicken with impunity. Of the 4 who have discontinued antigen avoidance, one is now free of dermatitis altogether and the other 3 can now tolerate all the previously excluded foods.

It is often suggested that these diets are too difficult for patients and their parents. Of the 36 children entering the study 9 were excluded from analysis for non-adherence to diet, but in only 2 of these did this occur during the trial diet; if there is clinical response to dietary treatment the difficulties are cheerfully borne by the child and parents.

A further aim of this study was to assess whether a careful history, prick tests and the RAST could identify those children most likely to benefit from dietary treatment. At entry to the trial we sought a history of symptomatic food allergy. Only 4 of the 20 completing patients gave a history of cutaneous reactions to foods and in only 1, possibly 2, was this an eczematous reaction. There was no association between positive prick tests to 5 egg and cows' milk preparations (whole egg, egg yolk, egg white, β-lactoglobulin, α-lactalbumin) and response to dietary avoidance (Fig. 1). We also did the RAST, using 5 egg and cows' milk antigens: ovalbumin,

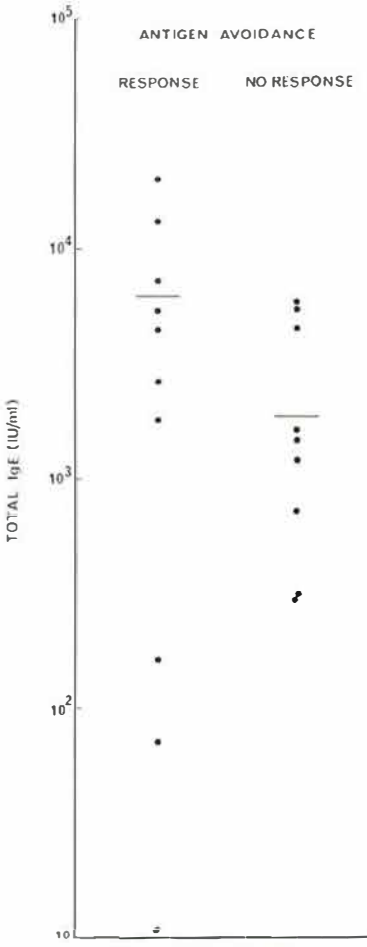

Fig. 3. Serum total IgE levels before antigen avoidance according to subsequent clinical response.

bovine serum albumin, β-lactoglobulin, bovine gamma-globulin and α-lactalbumin. All except one patient had at least one positive test. There were more positives in those patients who showed a good clinical response to antigen avoidance (Fig. 2); as anticipated, the mean serum IgE was also some-

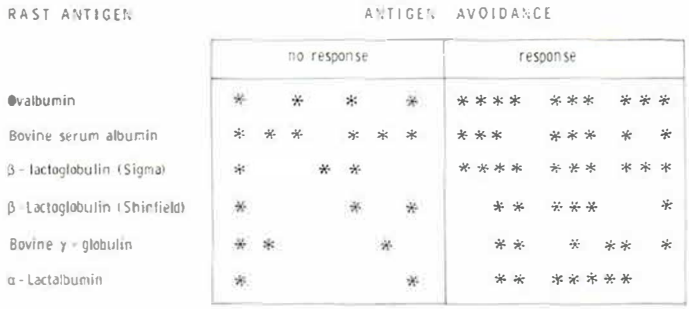

Fig. 2. Positive RAST (*) to egg and cows' milk antigens before dietary egg and cows' milk avoidance, according to subsequent clinical response. Vertical columns show results for individual patients. The assay uses microcrystalline cellulose particles; binding exceeding 1.6× cord serum value is taken to indicate positivity.

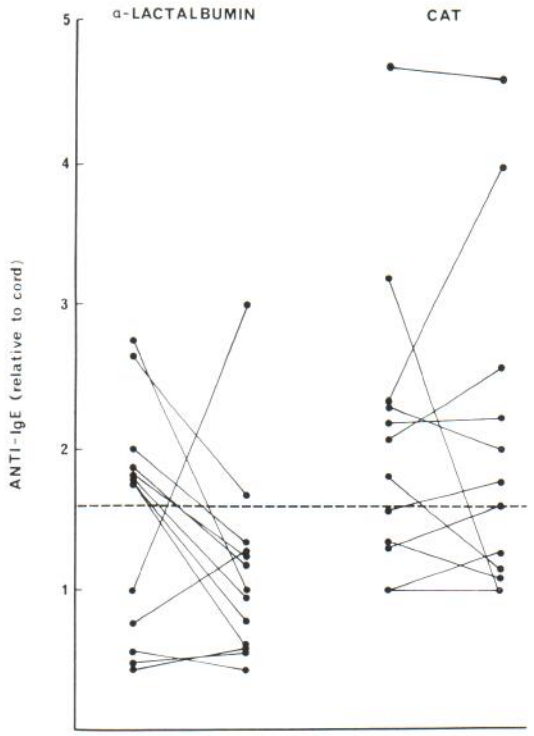

Fig. 4. Serum levels of specific IgE to α-lactalbumin and cat dander in a RAST assay using microcrystalline cellulose particles. The broken horizontal line indicates our laboratory threshold for positive binding. Values are shown both before a and after 4 weeks' dietary egg and cows' milk avoidance in individual patients.

what higher in this group (5 280 IU/ml compared to 1 872 IU/ml, Fig. 3); neither of these differences reached statistical significance.

The specific IgE antibody assays were performed on sera collected at the start and finish of the first dietary period, whether 'trial' or control; in 13 patients this was the 'trial' diet period. In Fig. 4 the changes occurring in the serum levels of specific IgE to α-lactalbumin during this 4-week period are compared with those to cat dander. Sharp decreases in specific IgE to α-lactalbumin occurred in 8 of the 13 patients, and in 7 of these the test would have been reported as having changed from positive to negative. These changes approached statistical significance in the paired 'T' test (log transformed data, $0.05 < p < 0.1$). The changes in serum levels of specific IgE to cat dander show no discernible trend. In a few patients large and unexplained changes in specific IgE levels to both antigens were seen. These data suggest that avoidance of an allergen can induce appreciable changes in serum levels of specific IgE antibody in a surprisingly short period of time.

Thus, neither history, prick test nor the RAST would have enabled us to identify those children most likely to benefit from dietary avoidance of egg and cows' milk antigens. We considered that the evidence was strong for benefit from such a diet. The problems remained the selection of children for such treatment, and the identification of the relevant dietary allergens upon which to base antigen avoidance diets in the individual case. Clearly we had only considered a few foods in this initial study; presumably other food allergies were present, particularly in those children who did not show dramatic responses to avoidance of cows' milk, egg, beef and chicken alone. We decided that the absence of more sophisticated tests for allergy diagnosis would force us to elucidate the individual patient's allergies on a basis of trail and error. This process would necessarily be laborious and timeconsuming for patient, parents, doctor and dietitian.

We have gained the impression that younger children are more likely to experience worthwhile benefit from dietary antigen avoidance, though the reasons for this are unclear. It is suggested that allergy to airborne allergens becomes increasingly important in the eczematous child with advancing age. Furthermore, the administration of demanding diets is accompanied by fewer problems in the pre-school child. We have now concentrated our attention on this group for a further study. Although the progress of this study is still at an early stage I would like to describe briefly what happens to these patients in our hospital. The opportunity to investigate the child's food allergies in more detail is offered to parents of pre-school children if the response to general measures, ordinary topical treatment and a standard egg and cows' milk avoidance diet is judged inadequate. The patient then progresses through 3 distinct phases. Initially he is

Table I. '*Oligo-antigenic*' *diets*

1	2
Lamb	Turkey
Rice, rice flour etc.	Potato, potato flour
Carrots, swede	Cabbage, Brussels sprouts
Goats' milk	Soya 'milk'
Apricots	Peaches

put on what we have optimistically called an 'oligo-antigenic' diet. There are 2 such diets, each entirely different (Table I). Diet 1 is given first, for a period of 4 weeks. If marked improvement occurs, the patient will be able to progress to the second phase in which foods are re-introduced on a scheduled basis. If marked improvement does not occur, the patient is given diet 2 for a 4 week period, to rule out the possibility of allergy to any constituent of diet 1. If a child does not benefit from either diet, food allergy is unlikely to be a major aetiological factor in his particular case. During the reintroduction phase one new food is tried each week. The food is given on 4 successive days, a very small quantity on the first day, then a larger quantity on the next 3 days. If no adverse reaction is noted during these 4 days or during the 3 subsequent days, this food can be added to the basic diet and eaten freely. If any reaction is noted, particularly a cutaneous reaction, the parents note the details in a special diary and the food is not given again. There are of course special problems with certain foods containing several constituents, such as bread, which may include wheat, soya, yeast and often also milk and pork fat. With such foods we specify particular recipes or commercial brands whose contents are known, and reintroduction is not attempted until each constituent has already itself been successfully reintroduced. At the end of several months, each patient should have identified a list of foods to which allergy is suspected. We are keen to attempt confirmation of these allergies by double-blind challenges; this is the purpose of the third phase. H. J. Heinz & Co. have very kindly made up suitable preparations for these challenges. There are 2 'carriers', one savoury, based on carrots and lentils, and one sweet, based on apricots and rice. The carriers themselves are used for the control challenges and a variety of foods such as wheat, egg, milk, chicken and pork are added to these in such a way that identifiable alteration is minimised. For each 'true' challenge a control challenge is given. One challenge is given each week, in hospital; the order is randomly allocated by the dietitian. Each challenge is given over 7 successive days, a small quantity only being given on the first day. Insufficient data are available at this stage to justify discussion here, but we believe that successful allergy diagnosis can be achieved using these methods. One hopes, though, that better understanding of the immunopathogenesis of atopic dermatitis will lead to a more fluent approach to the identification and treatment of patients' allergies.

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DISCUSSION

Rook (Cambridge). Q: Children when they first start to feed themselves are not very good at getting food into their mouths—they get it all over their hands and all over their faces. What is your view as to the importance of contact urticaria to foods as a mechanism by which foods exacerbate eczema in small children?

A: I believe that this does happen. Similarly, when food and other antigens exacerbate eczema following systemic absorption, the initial lesion may always be urticarial. Eczematisation might then follow if scratching occurs.

Concluding remark

By Kjell Aas

I would like to finish off this session with what I believe to be very important. We must be aware that technical details play an important role in establishing allergy. The RAST classes 1 and 2 are often non-specific. One can have non-specific reactions also to skin tests. When using a commercial extract in a given concentration one can have 30–50 % reactions which are called positive, but if one dilutes that extract twenty times, it will then give a 95 % correlation. Furthermore, we must not forget the nature and the natural causes of this disease. It is fluctuating and multifactorial, so we need very strictly controlled trials without compromise.

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MONOCYTE CYTOTOXICITY IN CLINICAL EXACERBATION AND REMISSION OF ATOPIC DERMATITIS

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Abstract. Six adult patients with severe atopic dermatitis were followed up for an average time of 9 months, through periods of clinical exacerbation and remission. Sequential studies of monocyte function measured as antibody-dependent cell-mediated cytotoxicity showed depressed function which did not normalize during clinical remission.

Key words: Antibody-dependent cell-mediated cytotoxicity; Monocytes; Atopic dermatitis; Remission; Exacerbation

Several studies have suggested immunologic dysregulation in patients with atopic dermatitis. However, only a few sequential studies have been performed. In these studies there has been a lack of agreement over the correlation of the clinical state with serum IgE levels (4, 9). For a decreased lymphocyte transformation test, no influence of the clinical state was noticed (1), while for polymorphonuclear leukocytes, chemotaxis normalized following clinical remission (7).

Earlier we showed that monocyte function measured as antibody-dependent cell-mediated cytotoxicity (ADCC) was reduced in patients with severe atopic dermatitis during exacerbation of their disease (2). The purpose of this investigation was to determine by sequential studies on individual patients whether or not monocyte ADCC fluctuated with exacerbations and remissions in skin involvement.

MATERIALS AND METHODS

Patients and controls. Six patients with recurrent widespread dermatitis were included. Their ages ranged from 21 to 33 years (median 24). Two of them had in addition asthma, though not during the observation period. None had received systemic glucocorticoids.

Patients were observed over an average period of 9 months. The first blood sample was taken during acute widespread dermatitis and repeated when there was no sign of dermatitic activity. Between these two samples the patients were treated with potent topical glucocorticoids and/or tar. After remission, blood sampling was repeated every second months over a period of 6 months and on the same occasions the extent and severity of the disease were graded according to Rogge and Hanifin (7). During this period all patients experienced some fluctuation in their disease state, but the treatment was limited to topical hydrocortisone or hydrocortisone-butyrate from time to time. Antihistamines were withheld 72 hours before every blood sample. Controls were 36 healthy volunteers, their ages ranging between 17 and 55 years (median 30).

Preparations of monocytes. Heparinized venous blood (20 I.E. preservative-free heparin/ml) were spun over Ficoll-Isopaque as described by Böyum (4). The resultant interface layer, which contained the mononuclear cells, was washed twice in Hanks' balanced salt solution (HBSS) with 2.5 % (v/v) heat-inactivated fetal calf serum (FCS), (Gibco, Grand Island, N.Y., USA). The washed cells were resuspended in medium RPMI 1640 with 25 mM HEPES (Gibco) supplemented with 25 % (v/v) FCS and dispensed to tissue culture flasks (Nunc, Roskilde, Denmark). After incubation in a humidified atmosphere containing 5 % CO_2 for 1 h at 37°C the non-adherent cells were decanted and the flasks rinsed with 3 changes of HBSS with 2.5 % FCS at 37°C, leaving behind the adherent monocytes. After introducing HBSS with 2.5 % FCS into the flasks they were placed on an ice bath for 30 min. Detached monocytes were then decanted and washed once. Finally, the cell concentration was adjusted to 1×10^6/ml. Judged by non-specific esterase activity in cytocentrifuged preparations (33) the median percentage of monocytes was 91, ranging from 82 to 96. 0–3 % granulocytes were found. The other contaminating cells were lymphocytes. As assessed by trypan blue exclusion, the viability was always higher than 95 %.

Labelling of erythrocytes. Equal volumes of washed type B human erythrocytes (200×10^6/ml) and sodium ^{51}chromate (Radiochemical Centre, Amersham, England; 1 mCi/ml, 2–10 μg Cr/ml) were mixed and incubated for 60 min at 37°C. After labelling the erythrocytes were washed twice.

Antiserum. Human hyperimmune antiserum to type B human erythrocytes was obtained from the Blood Bank and Blood Grouping Laboratorium, Aarhus Kommunehospital. Serum was heat-inactivated and stored at −20°C. A dilution inducing maximal lysis was used.

Cytotoxicity assay. The tests were set up in duplicate in round-bottomed plastic tubes. All dilutions were made in medium RPMI 1640 supplemented with 25 mM HEPES, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 5 % FCS, finally adjusted to pH 7.4. To 100 μl monocytes was added 100 μl ^{51}Cr-labelled erythrocytes (1×10^6/ml and 8×10^6/ml and 100 μl antiserum dilution.

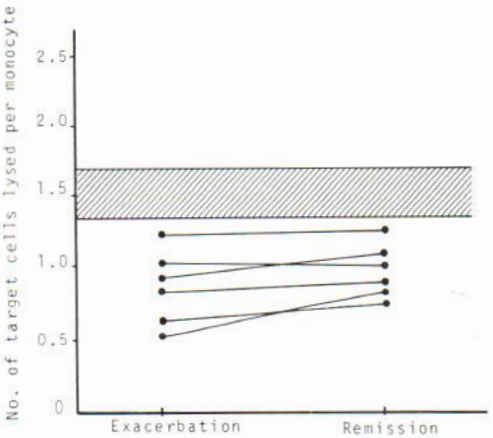

Fig. 1. Monocyte ADCC in patients with atopic dermatitis at time of clinical exacerbation and remission. The lines connect the paired results from each person. The hatched interval marks the 95% confidence limits for normal individuals. (●) denotes patients with atopic dermatitis.

Controls in which the monocytes were replaced by unlabelled erythrocytes (1×10^6/ml) were included. The mixtures were centrifuged at 160 *g* for 1 min and incubated in a humidified atmosphere containing 5% CO_2 at 37°C. After 1 h and 18 h the tubes were centrifuged at 300 *g* for 10 min. Half of the supernatant was withdrawn. Both this supernatant (S) and residues (R) were counted separately in a well-type gamma counter. Cytotoxicity was calculated as:

$$S \times 2/(R+S)$$

By subtracting the release in control tubes the specific cytotoxicity (C) was defined. Results were expressed as the number of erythrocytes (E) lysed per monocyte (M):

$$E \times C/M.$$

RESULTS

During clinical exacerbation, monocyte ADCC was invariably decreased (Fig. 1). In clinical remission, the function in 5 cases increased slightly without reaching normal values. In the last case a minor fall in cytotoxity was observed from exacerbation to remission.

Between exacerbation and remission, patients were treated with potent topical glucocorticoids. To answer the question whether this treatment had maintained impaired function even though clinical remission had occurred, 4 out of the 6 patients were observed for a further period of 6 months (Fig. 2). During this period some fluctuations of disease activity were observed, but patients were only from time to time treated with topical glucocorticoids of low potency. As illustrated, only minor fluctuations in monocyte ADCC occurred. Only for one patient did the function normalize, but on the following occasion this patient again displayed depressed function. There was no significant correlation between changes in monocyte function and the clinical state of the disease.

DISCUSSION

Because of the rapid fluctuations which may be seen in the severity of lesions in patients with atopic dermatitis, we chose to observe individual patients through exacerbations and remissions. Our observations suggest that decreased monocyte ADCC tends to remain low in quiescent phases of the disease. Even though potent topical steroids were used only during exacerbation, it could not be ruled out that concurrent therapy was responsible for the hyporeactivity. Besides, it was not possible to say from this investigation, if or when normalization would take place if the patients had been free from dermatitis for longer time.

In spite of the rapid turnover of human monocytes which under normal conditions leave the vascular space with a half-time of about 8 hours (5), it has been shown that suppression of normal monocytes following thermal injury can be prolonged. In patients with mild or moderate burns the phagocytic functions returned to normal after 3 to

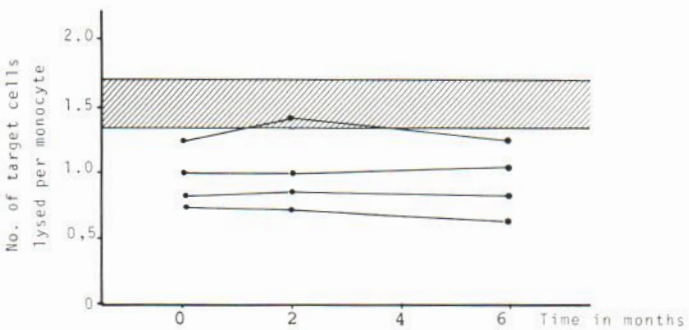

Fig. 2. Individual variations in monocyte ADCC in atopic dermatitis. The lines connect results from one person (●). The hatched interval marks the 95% confidence limits for normal individuals.

6 weeks, but in the group with severe burns, the dysfunction persisted after 7 weeks (3).

There has been some controversy over the relationship between altered immune reactivity and the clinical state of patients with atopic dermitis. Sequential estimation of total levels of serum IgE and specific IgE has shown no significant correlation between IgE levels and the severity of skin involvement (4, 9), but in another report some correlation was shown (6). For lymphocytes, a decreased number of circulating T lymphocytes and hyporeactive lymphocyte transformation test to PPD and herpes simplex antigen have been found during both exacerbations and remissions (1). In serial comparisons of PMN chemotaxis in 4 out of 4 patients with erythrodermal atopic dermatitis a dramatic parallel with clinical improvement was found (7). The same patients also expressed decreased monocyte chemotaxis during exacerbation, but monocytes were not tested again in quiescent periods. Impaired monocyte chemotaxis has also been found by another group of investigators, and they were not able to correlate this finding to the severity of the dermatitis. However, the individual patients were studied on one occasion only (8).

It is difficult to compare our results with other sequential studies, because different cell types have been tested in different assay systems. As mentioned, serum IgE also tends to remain abnormal in clinical remissions. Nevertheless, a common factor behind elevated IgE levels and low monocyte function is unlikely, as depressed monocyte ADCC is not correlated to serum IgE levels (in preparation).

The association between severe atopic dermatitis and monocyte function remains speculative. However, the fact that monocyte ADCC remains depressed despite fluctuations in the clinical state could suggest that a defective monocyte function is part of a constitutional basis for atopic dermatitis.

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RAST WITH HUMAN DANDER ALLERGEN IN ATOPIC DERMATITIS

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Abstract. Using highly purified allergens from skin flakes of the human scalp and from house dust, it was demonstrated that IgE antibodies against the former can be detected only in cases of severe atopic dermatitis (AD), but not in asthma (without eczema) and not in hay fever or rhinitis patients. The RAST scores for human dander correlated well with total IgE levels. Also, RAST scores for human dander allergen correlated very well with those for house dust. This could not have been due to allergenic cross-contamination, as the house dust RAST can be strongly positive in asthma and rhinitis patients, whereas the human dander RAST is always negative. Haemolytic complement consumption by both allergens, in contrast, correlated well in all these diseases. The results indicate a dissociation of basic mechanisms in asthma versus AD.

Key words: RAST: Human dander allergen; Atopic dermatitis

The allergen in skin flakes of the human scalp has long been associated with the atopic condition, most particularly with atopic dermatitis (AD) (7, 8, 9, 10, 11). In such patients, and in subjects with bronchial asthma, immediate-type skin reactions with aqueous human dander extracts are observed very frequently (7, 9, 11, 12, 14). Several authors have reported the successful transfer of this reactivity by way of the Prausnitz-Küstner reaction (8, 9, 11). Although this suggests mediation by reaginic antibody, no reports are available so far on the existence of IgE antibody against human dander components. In an unspecified group of sera of allergic patients, Brighton & Topping (5) reported very low RAST-scores with an extract of skin (not from the scalp). The present paper records our experience with human dander and house dust RAST in patients with atopic dermatitis, as opposed to bronchial asthma or other allergic disease.

MATERIALS AND METHODS

Patients and sera. A group of well-registered patients of the Dermatology Ward, with widespread AD of lung duration, was recalled for investigation. Patients with bronchial asthma, hay fever or vasomotor rhinitis were selected from subjects attending the Out-patient Department of Clinical Allergy. Venous blood samples were allowed to clot in glass and the sera were stored at −70°C until use.

Allergen and IgE-determination. House dust allergen was extracted from a pool of dust and purified to the stage of fraction E, as described elsewhere (1). Human dander allergen was obtained from acetone-washed human scalp scales and purified to the fraction E stage by a previously published schedule (1). The purified allergens were coupled to cellulose discs with cyanogen bromide *ad modum* Ceska, Eriksson & Varga (6). Radiolabelled anti-(Dε2)IgE antibody was purchased from Pharmacia Diagnostics, Uppsala. Total IgE was determined by RIST (Pharmacia reagents).

RESULTS

In previous communications (1, 3), we have reaffirmed the well-documented observation of a close correlation in the incidence of positive immediate-type skin reactions to house dust and human dander in atopic patients in general (7, 8, 9, 13, 14). Despite this association of reactivities in unselected groups of atopic subjects, no such correlation was found between house dust and human dander RASTs in the blood sera (3). The cause for this becomes evident on reviewing the results summarized in Figs. 1 and 2.

Fig. 1 shows that the human dander RAST was not found positive in patients with asthma or rhinitis uncomplicated by eczema; in such patients, only the house dust RAST was positive. Positive human dander RASTs were obtained only in cases of (severe) AD, as shown in Fig. 2. The data in Fig. 2 also demonstrate a close correlation in AD between the RASTs for house dust and human dander allergen.

In AD in adult patients, the probability of a positive human dander RAST increased with total IgE levels. As depicted in Fig. 3, there was a good correlation in this respect between RAST and RIST values, as has been reported before for RIST and house dust RAST (4).

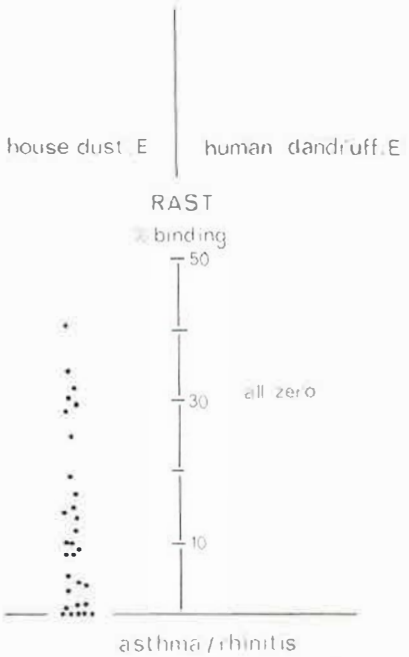

Fig. 1. RAST with purified house dust and human dander allergen in a group ($n=30$) of atopic patients with bronchial asthma or vasomotor rhinitis and with positive skin reactions to both allergens. RAST results expressed in % radioactivity bound to the discs from a standard incubation dose.

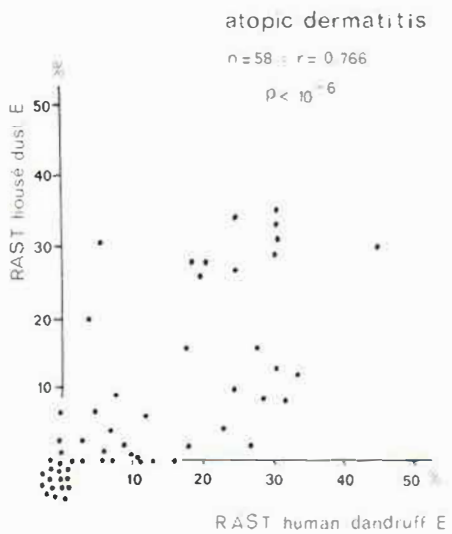

Fig. 2. Correlation of house dust and human dander RAST in the sera of patients ($n=58$) with atopic dermatitis. Spearman rank correlation coefficient $r=0.766$ ($P<10^{-6}$).

In the adult dermatitis group, very high RIST levels tended to be the rule ($n=66$, geometric mean IgE = 1 323 IU/ml), in association with positive human dander RASTs. In children with AD, below the age of 10, RIST values were lower ($n=12$, geom. mean IgE = 655 IU/ml) and the human dander RAST was frequently negative.

DISCUSSION

Immediate-type skin reactions to house dust and human dander allergen frequently run parallel (1, 3, 7, 8, 9, 13, 14). This is also true for the *in vitro* complement consumption with both allergens in various blood sera (3). In an unselected group of atopics, no parallelism was found in specific IgE antibody to both allergens (3). The present paper documents that this discrepancy is due to the fact that the RAST human dander is positive only in cases of AD, but not in bronchial asthma, hay fever or rhinitis. Figs. 1 and 2 clearly demonstrate that cross-contamination of the purified allergens had not occurred.

The data demonstrate a dissociation in the pattern of reactivity of the house dust and human dander allergens. The results of Young (13), Young & Bangma (14) and of French authors (7, 9) are relevant in this context. Despite parallel skin reactions to both allergens in asthma or atopic eczema,

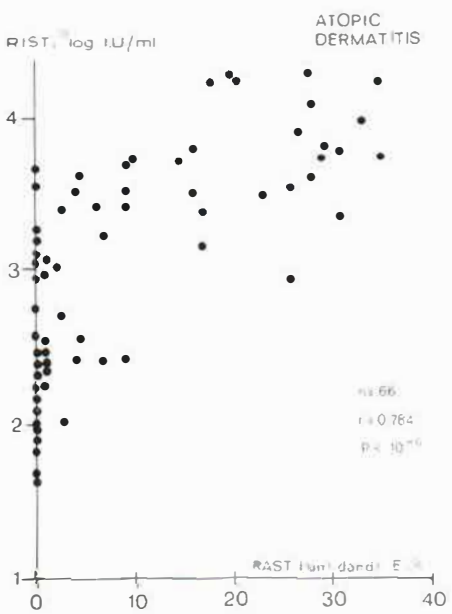

Fig. 3. Relationship between total IgE levels (RIST, in IU/ml) and human dander RAST scores in 66 patients with atopic dermatitis. Spearman rank correlation coefficient $r=0.784$ ($P<10^{-6}$).

inhalation-provocation tests with the dander allergen tended to be positive only in the eczema group, but were negative in the bronchial asthma patients. Current studies aim at further defining the precise role of the IgE antibody in these phenomena.

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The authors are indebted to Dr W. J. Koers for selecting the patients and providing the clinical data.

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DISCUSSION

Saurat (Paris). Q: Have you made electron microscopical studies of your starting material in preparing human dandruff allergens? Among others, you can have microbial products and yeasts in this material, so that the composition of such an antigen would be very broad.

A: I think the point is well taken, but we have no electron microscopic, not even microscopic studies of this. We take the pragmatic view that we use extracts as they are being used in practice by clinical allergists. I doubt, however, that this reactivity could be due to contamination to either moulds or bacteria, because we have been investigating a whole range of yeast extracts, etc., and none of these antigens has the extreme reactivity that the human dandruff antigen has. I cannot exclude the point, but I think it is very unlikely that it is contaminated by another antigen.

Zachariae (Aarhus). Q: What about mites?

A: We did not look for mites either, but we have investigated a whole series of commercial mite allergens and some we prepared ourselves, and none of these has the reactivity that the human dandruff antigen has.

Hanifin (Portland). Q: We discussed the possibility that staphylococcus may play a role in the etiology of atopic dermatitis. Have you done any qualitative studies with staphylococcal reactivity? I am also curious as to whether these patients react to human saliva.

A: I do not know of any studies where human saliva has been tested intracutaneously or otherwise in vivo. We have tested the reactivity of human saliva in vitro, but it is far from reaching the reactivity that one can solve with human dandruff. Human saliva does not react at all with complement. We have also investigated various extracts of *Staphylococcus aureus* and this does not run parallel with human dandruff extract. I think the reactivity of the human dandruff allergies has to do with decomposition reactions of the skin components themselves. Simon has proved that reactivity is really to be found in the scalp scales. We have recently investigated a preparation that was made of skin flakes not obtained from the scalp but this did not have any reactivity at all. I think that there is no evidence that this reactivity had to do with bacterial extract, with saliva, with yeast or moulds, but has to do with the decomposition products which arise in the human corneum itself.

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PRURIGO REACTION IN ATOPIC DERMATITIS

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Abstract. A total of 41 biopsy specimens of the first visible prurigo papule were obtained from 32 adult patients with atopic dermatitis. In 38 of the 41 biopsy specimens, histological changes were seen in connection with hair follicle. The follicular wall showed spongiosis and vesicle formation with mononuclear cell migration. The remaining 3 biopsy specimens revealed an eczematous change which involved the surface epidermis. Multinucleated epidermal cells were observed in 34 of the 41 biopsy specimens. The giant cells occurred at skin sites of eczematous inflammation.

Key words: Prurigo reaction; Spongiosis; Multinucleated giant cells.

Skin manifestations of atopic dermatitis are usually classified into three types of reaction: eczematous reaction, lichenification, and prurigo reaction. The eczematous lesions and lichenified patches of atopic dermatitis may occur in any part of the body, including the palms and soles. The prurigo lesions of this condition, however, do not develop in the palmo-plantar skin areas. The anatomical basis for the absence of prurigo lesions in palmo-plantar skin is unknown.

It is interesting to note that the palms and soles are also intact in prurigo simplex subacuta, which is a representative of subacute to chronic forms of prurigo diseases. Some reports (1, 2, 5) suggest that the early changes of prurigo simplex subacuta may occur in connection with hair follicles.

The main aim of the present study was therefore to determine whether or not the primary changes of prurigo lesion in atopic dermatitis begin to take a follicular pattern.

MATERIALS AND METHODS

Selection of patients. A total of 32 adult patients with atopic dermatitis were selected for this study. They had lichenified patches in the flexor aspects of limbs and prurigo lesions on the trunk or extensor surfaces of extremities. They were healthy except for the pruritic skin disease.

Clinical observations. In all patients, the first discernible lesions of prurigo reaction were normal colored or slightly reddish papules 1–2 mm in diameter. These prurigo papules were deep-seated in the skin, and were recognized more easily by palpation than by inspection.

Histological studies. A total of 41 biopsy specimens of first visible prurigo papules were obtained. 30 specimens were taken from the lesions on the extremities, and 11 specimens were from those on the trunk. To visualize three-dimensionally the histological features of the early prurigo papule, all biopsy specimens were serially sectioned and stained with hematoxylin-eosin.

RESULTS

In 38 of the 41 biopsy specimens, the histological changes occurred in connection with hair follicles. The follicular epidermis showed spongiosis and vesicle formation with mononuclear cell migration. The dermis around the involved follicle revealed perivascular infiltrates of mononuclear cells. Sweat ducts and the surface epidermis far from the affected follicle were intact. In the remaining 3 specimens, spongiosis and migration of mononuclear cells were seen at the surface epidermis. Thus, in all specimens examined, the histological change was eczematous in nature.

Multinucleated epidermal cells were frequently found in the involved follicular epidermis and in the adjacent surface epidermis. The epidermal giant cell had 3 to 10 nuclei. Intercellular bridges were clearly demonstrated between giant cells and neighboring prickle cells.

DISCUSSION

This study demonstrated that the majority of the first discernible prurigo papules of atopic dermatitis follow a follicular pattern. The papules were deep-seated in the skin. Histologically, they showed an eczematous change which involved the follicular epidermis.

Some investigators (4) consider that the first-visible papular lesion of atopic dermatitis implies more than epidermal involvement, simply because the surface epidermis above the papule is intact. They then adhere to the view that the corium is the

most likely site of the primary lesion of atopic dermatitis. However, to my knowledge, they do not perform histological examinations of the first visible eruption.

From the results of the present study, it seems most reasonable to consider that the first-visible prurigo papule of atopic dermatitis is deep-seated because an eczematous change occurs at the site of follicular epidermis. This hypothesis may account for the absence of prurigo lesions in the palmoplantar skin areas where there are no hair follicles.

The present study also demonstrated multinucleated epidermal cells in the involved follicular wall and in the neighboring surface epidermis. The nuclei and cytoplasma did not have the bizarre appearance of the giant cell in viral skin diseases and in malignant skin tumors. The multinucleated epidermal cells resemble those seen in parapsoriasis guttata and eczematous dermatitis (3).

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DISCUSSION

Scarpa (Trieste). Q: Are the multinucleated epidermal cells you show in these patients really multinucleated, or are they only group cells? Have you done ultrastructural studies?

A: We have not done electronmicroscopical studies. Quite similar epidermal cells have been described in parapsoriasis guttata and various eczematous conditions.

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SEASONAL FACTORS IN ATOPIC DERMATITIS AND THEIR RELATIONSHIP TO ALLERGY

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Abstract. The number of first visits to an out-patient department by patients with atopic dermatitis, as well as anamnestical data from such patients, clearly shows seasonal changes in the course of this disease. An obvious relation is demonstrable between seasonal exacerbations and allergy to seasonal allergens.

Key words: Atopic dermatitis; Seasonal factors; Relation to allergy

It is well known that there are seasonal changes in the course of atopic dermatitis, many patients showing a tendency to improve in summer and to deteriorate in winter. According to Schnyder (3) and Rajka (2) only 10% of all cases fail to show any seasonal dependence. It was suggested by Pirilä (1) that in winter, the shorter duration of daylight plays the principal role in this deterioration.

According to Schnyder (3) house dust cannot be incriminated, for the exacerbation in winter would be more common in cases in which skin tests with house dust are negative than in those with positive reactions. Although several factors may be responsible for improvement or deterioration in summer there was some evidence, according to Rajka (2), that reactivity to summer pollens was a little more common in patients who deteriorated compared with those who improved, in summer. The incidence of sensitivity to spring pollens was also found higher (by Rajka) in patients deteriorating in spring, than the overall incidence of pollen sensitivity.

Table I. *First visits to the out-patient department over a 4-year period*

Season	Number of first visits	Percentage of patients
Spring	35	21
Summer	34	20.5
Autumn	62	37
Winter	36	21.5
Total	167	

METHODS AND RESULTS

In order to investigate further these possible relationships, we noted the first visits to our out-patient department of patients with atopic dermatitis over a 4-year period. The findings are given in Table I.

In Table I a striking preference is apparent of first visits to the autumn, supposed to correspond to a preference of complaints in that season. To investigate this possibility, we questioned 106 patients with atopic dermatitis about possible seasonal susceptibility to complaints. The results are given in Table II.

From the data given in Table II it seems probable that indeed generally there is a tendency to seasonal susceptibility to complaints in the autumn, though a minority of patients still had a tendency to suffer more complaints in spring and summer.

If we call the former group of patients "the autumn group" and the latter "the spring and summer group" and we look for a possible relationship of these groups to the allergy pattern in regard to reactions to house dust or pollen, we come to the results given in Table III.

A statistically significant relationship exists between seasonal susceptibility to complaints and allergy pattern, as given in Table III.

Table II. *Seasonal preference of complaints (anamnestical data)*

Season	Number of patients	Percentage of patients
Complaints mainly in autumn	28	26.3
Complaints mainly in spring+summer	17	16
Complaints mainly in other seasons	14	13.2
Seasonal preference	59	55.5
No seasonal preference	47	44.5
Total	106	

Table III. *Seasonal susceptibility to complaints in relation to allergy*

(*a*) Complaints mainly in autumn ("autumn group")	28 patients		
allergy pattern:	house dust +	27 patients	pollen + 2 patients
	house dust −	1 patient	pollen − 26 patients
(*b*) Complaints mainly in spring and summer ("spring and summer group")	17 patients		
allergy pattern:	house dust +	5 patients	pollen + 6 patients
	house dust −	12 patients	pollen − 11 patients

DISCUSSION

Of course it needs no explanation why allergy to pollen must be of significance as regards complaints in spring and summer, but perhaps it could be more difficult to explain a relationship between allergy to house dust and exacerbation in the autumn.

For this we should like to refer to an investigation by Voorhorst in asthmatics (4).

He found that the first visits of asthmatic patients with allergy to house dust show a striking preference to the autumn, whereas no such preference is to be seen in asthmatics without allergy to house dust. Because of this, in asthma a distinct relationship between house dust allergy and a predominance of complaints in the autumn appears to be present. In our investigation the same seems to be the case in atopic dermatitis.

CONCLUSION

Because of all these facts we must conclude that there is certainly a relationship between seasonal susceptibility to complaints and allergy to seasonal allergens, which points to the fact that allergens must play a role in several cases of atopic dermatitis.

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DISCUSSION

Zachariae (Aarhus). Q: When does autumn start in Holland?

A: I think we can best state September.

Zachariae: We have always said that our atopics in Denmark get worse in winter, but this should be later in the autumn.

Berrens (Utrecht). Q: Have you compared the percentage distribution of the number of patients visiting the outpatient department with atopic dermatitis with the total number of dermatological patients visiting the clinic with any dermatological disease?

A: Yes we did that.

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THE NATURAL HISTORY OF ATOPIC ECZEMA

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Abstract. A long-term follow-up study of 2 000 children with atopic eczema for from two to twenty-one years: clearance rates, pubertal recurrence rates and factors with or without prognostic significance are reported. Late onset, "reversed pattern" and possibly social factors are adverse features, whilst early onset, seborrhoeic pattern and male sex are favourable prognostic signs. These results are based on a follow-up rate better than 90 % and are the first results in the literature of a prospecitive survey of this disease.

Key words: Atopic eczema; Prognosis; Clinical patterns

The prognosis of infantile eczema is always said to be good but the published literature is at variance with this (1, 3 and 5), most authors quoting 40–50% recovery at 15 years. In a 15-year follow-up study in Sunderland, 60% of patients with eczema had persistent trouble at 15–17 years (2). These, and other, studies all suffer from one of two major criticisms: either they were dealing with in-patients and/or they had very low follow-up rates (30–50%).

A follow-up study of 256 out-patient cases of infantile eczema in 1956 (4) showed a very favourable prognosis, based on a 95% follow-up rate.

Following that study, since 1958, a prospective survey of all cases of infantile eczema seen as Out-patients within six months of diagnosis was undertaken. Cases admitted subsequently remained in the follow-up study and no child was discharged from follow-up.

The age range in 1979 is 4–26 years. Some of these children have been followed for 20 years, but the majority for a considerably shorter period (Table I). The follow-up rate has remained remarkably high, probably because this is a prospective survey and every patient, or his/her parents, was told that they were in the series and was asked to advise any change of address. The follow-up rate in 1978 was 97% at 5 years and 95% at 20 years, although the 20-year follow-up was only on a relatively small number of patients (259).

The most important single factor which we were attempting to determine in this study was the true recovery rate of children with atopic eczema and Table II shows the clearance rate at various periods of time. It also shows the recurrence rate—it is a well-known fact that some children will clear at the age of a few months or, perhaps, within a year or two, only to relapse either later in the childhood period or at puberty.

The next aim of this study was to determine the factors influencing the prognosis. Those factors with no influence on the prognosis include the severity of the disease at onset, the position of the child in the family, the method of infant feeding, and concomitant ichthyosis.

Prognostic factors of great significance include: age at onset and the so-called "reverse pattern" of infantile eczema. If we take age at onset first and study the clearance rate at various periods of time with various ages at onset, there is little difference in the prognosis for children whose eczema starts before 6 months vis-à-vis 6–12 months. There is a suggestion of a worse prognosis for children whose eczema starts between 12 and 24 months. The prognosis for children whose eczema starts after the age of 2 years is considerably worse (only 50 % clear at 10 years). The influence of family size on the prognosis is difficult but there is a strong suggestion from the data that the only child has a worse prognosis than a child in a large family.

Clinical findings

During this study, a hitherto clinically unrecognised pattern of infantile eczema has become apparent, which has been termed the "reversed pattern". In this condition, the child develops eczema not only in the ante-cubital and popliteal fossae, but also on the knees and elbows, often with lesions on the dorsum of the wrists and hands. If one looks at the prognosis for these children (Table III) (there were 94 in my series), the prognosis was considerably worse.

Associated disease

There appears to be a slight worsening of the prognosis when the disease is associated with classical bronchial asthma, whereas allergic rhinitis and urticaria, when associated, do not appear to influence the prognosis at 10 years.

Favourable prognostic signs include seborrhoeic eczema of infancy and there is a clear favour for boys rather than girls.

Table 1. *Periods of time during atopic dermatitis patients have been followed*

No. of years	No. of patients
5	1 897
10	1 410
15	698
20	259

Table II. *Clearance rate of atopic dermatitis patients at various periods of time*

No. of years	Clearance (%)	Recurrence (%)
5	87	4
10	91.7	5
15	90	8
20	89	9

In summary, it is clear that when taking children referred to an out-patient clinic the prognosis of infantile eczema is as favourable as we have always suspected. The reasons for the differences between this study and all previously published ones are two-fold:

First, the failure to follow-up every single child: in my study in Sheffield (4) in 1968, of those children who replied to the first questionnaire, only 50% were clear. Of those who replied to the second, 88% were clear and of those who had to be asked three or four times, or more, 100% were clear, some of them not even realising that they had, in fact, had eczema as a child. Thus, any follow-up study on a retrospective basis is inaccurate.

The study described here is a prospective study and is potentially likely to give more accurate information. It may be argued that the prognosis for these children may have been modified by the active interest taken in them by a dermatologist. If that is so, it argues in favour of the contention that the prognosis is modified by treatment and that it has improved with more active treatment, notably with topical corticosteroids.

Another reason for the improved figures in this study when compared with others may be that all children who were admitted to this study were seen within 6 months of the onset of their skin disease and treated actively. Other studies have included all children referred to out-patient departments, many of whom have been troubled throughout their lives.

The clinical observation that the "reversed pattern" of eczema, along with a late onset, worsens the prognosis has not been reported before and in a new and retrospective study I have now examined 124 adult atopics with persistent eczema throughout their lives. Of these, 103 had either a late onset of their eczema, or the "reversed pattern". These facts suggest that the observations in the prospective study are, in fact, valid.

Table III. *Prognosis of atopic dermatitis patients with clinically "reversed pattern"*

No. of years	Reversed (94) (%)	
5	61	Clear
10	57	Clear
15	54	Clear

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DISCUSSION

Zachariae (Aarhus). Q: In your infantile eczema group, have you included infants with seborrhoeic dermatitis?

A: I have included in this group some 65 children with what would be described by some as infantile seborrhoeic dermatitis. Now, of these 65 the vast majority have cleared. In those whose affection has not cleared the dermatitis has slowly changed into straightforward infantile atopic eczema. I do not believe in infantile seborrhoeic dermatitis—I believe that infantile seborrhoeic eczema should be called a seborrhoeic pattern of infantile eczema.

Barnetson (Edinburgh). Q: Do you have any information in your patients on the prognosis of those who have concomitant food allergy, particularly to fish and to eggs, since these tend to be the patients with the highest IgE and with the more severe eczema?

A: I think around 6 or 7% of the children in this series had food allergy, and I think the prognosis for those children is worse. That study is not yet completed.

Q: Have you included in your material patients of families coming from India, Africa and so on and do you know anything about the prevalence of atopic dermatitis in these countries and what happens to them when they immigrate to Britain?

A: Quite surprisingly I haven't got many immigrants. Indians interest me intensely. They contract atopic eczema in the United Kingdom. They then go home for a prolonged holiday, 6 months a year, during which their skin clears entirely while at home, yet it recurs when they return to the UK. The incidence of atopic disease and atopic dermatitis, particularly in the West Indians, is very low and yet the incidence of atopic eczema in West Indian children in North West London is higher, much higher than expected. Again these children go home and the eczema clears. I can't give you any explanation.

Jones (Atlanta). Q: I would like to thank you, Dr Vickers, and congratulate you on the enormous job that could not

otherwise be done—at least not in America, because our population is so migrant. You show an 80–95% clearance rate within 5 to 10 to 15 years. Have you already selected the worst cases?

A: No, these are all general practitioner referrals. Those cases that are referred to me from pediatricians or allergists have nearly always had their disease for longer than 6 months, so they are automatically excluded. I have another study going on in cooperation with 3 general practitioners who are letting me look at the patients with infantile eczema, but whom they have considered not severe enough to warrant hospitalization. That study is now in its mid-term and these children have of course an incredibly much better prognosis.

Jones (Atlanta). Q: Might it be possible that your patients are not representative of all of the atopics born in your area, Liverpool, possibly because some of the more severe ones with asthma or bronchiolitis or whatever it might be are not coming to you?

A: I work in the largest children's hospital in Great Britain and I have a very close association with the respiratory physiologist who runs the asthma clinic, and I have looked at all the patients attending this clinic during the last 15 years. During all these years I only missed seeing a very small number, only a dozen in fact, who had atopic eczema.

Soter (Boston). Q: Some people claim that you shouldn't diagnose atopic eczema or infantile eczema in any individuals under 1 month, and I want to know how early you diagnose it. Secondly it has been claimed that episodes of acute urticaria are frequent with exacerbations of eczema and I would like to know how often you have seen this, as I have never seen it.

A: The only times I diagnose eczema of the infantile variety under the age of 6 weeks is when I diagnose the seborrhoeic pattern of infantile eczema. I have children who have an allergic reaction to food and develop urticaria and angioedema, and who within 3 to 7 days develop an exacerbation of their eczema. I am sure that it is in this way that food allergy is so very, very important in eczema—by the precipitation of attacks of urticaria or angioedema.

Rajka (Oslo): You asked whether the literature or you yourself were correct in stating the prognosis on atopic dermatitis. Since it was you who made the first reliable study, I am convinced you are right and we are very grateful to you. We cannot cure our patients, as we know, but we dare now say to all your patients and to the medical and non-medical press that the prognosis is not so bad as we earlier thought.

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EFFECTS OF HISTAMINE RECEPTOR ANTAGONISTS ON HISTAMINE-INDUCED RESPONSES IN HUMAN SKIN

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The effects of intradermally administered histamine H_1- and H_2-receptor antagonists on the cutaneous responses—redness, weal, flare and itch—induced by intradermal injection of histamine were studied in man. Wheal and redness were studied after blockade of the axon reflex by local infiltration with lidocaine. All responses were significantly inhibited by the H_1-receptor antagonist mepyramine. The H_2-antagonists cimetidine and metiamide reduced flare and itch significantly but not to the same extent as mepyramine and not in a clearly dose-related manner. The extent of wheal and redness was not significantly reduced by cimetidine. No further reduction of flare, itch or wheal was obtained by adding metiamide or cimetidine to mepyramine. After blockade of the axon reflex with lidocaine the histamine-induced wheals turned white at the centre. This blanching was more prominent when histamine was injected, in combination with cimetidine. Substituting mepyramine for cimetidine resulted in small wheals with an intense red colour. It is concluded that, apart from being engaged in the direct vasodilatory response to histamine, H_2-receptors do not seem to be involved in the other cutaneous responses to histamine studied.

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DISCUSSION

Q: Did you work with Dimaprit or any other histamine H_2-receptor agonist? I ask because we have used it and observed not only redness but also wheal and flare.

A: We had wanted to complete our investigation by studying the effects of some of these new H_2-receptor agonists, but we did not obtain permission to use them in humans. However, we tried the old drug betazole (Histalog) which has been used for gastric secretion studies for many years. I don't know how specific this drug is as an H_2-agonist, but it is known to stimulate gastric secretion without producing other histamine effects. Using low doses of betazole in the intradermal injections we obtained a redness but not the typical triple response. We thought this confirmed our results with inhibitors.

Zachariae (Aarhus). Q: Could not the decrease in the redness be ascribed to the edema of the wheal?

A: Wheal and redness were studied after blocking the axon-mediated flare reaction by local anaesthesia. Although the decrease in the redness appearing after cimetidine might seem subtle, our experiments were made in a double-blind fashion and I am quite convinced of our results.

Q: Could you be certain that your patients were certainly not atopics? Secondly, could atopics have different reactivity in and outside skin lesions and compared with normals? A: We have not injected histamine in skin lesions. We know from the studies of Georg Rajka that the itch duration is longer in atopics than in controls. Our own experience indicates that although the itch duration is longer in atopics, they do not show an increased flare reaction. I cannot exclude that some of our subjects could be classified as atopics.

Dobson (Buffalo) Some years ago we injected histamine into uninvolved surfaces of patients with atopic dermatitis and noticed greater wheals on flexural sites than on extensors, whereas this was not seen in normals.

Q: Have you any data that some antihistaminics are better than others? Hydroxizine is very effective in some cases of urticaria.

A: I cannot state that one antihistamine is better than another, but in this connection I might mention that some years ago we compared the effects of a Swedish antihistaminic drug, *N*-hydroxy-ethylpromethazine (Aprobit), which is a quaternary phenothiazine, with the tertiary derivative promethazine. We found that oral administration of Aprobit did not inhibit the cutaneous histamine responses (Acta Allergologica *29:* 462, 1974). It turned out later, that previous experiments had been performed after i.v. injection in animals.

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RECENT THERAPEUTIC EVENTS: CIMETIDINE® AND PUVA

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After the paper of Hägermark et al., including the experimental aspects of Cimetidine® as well as the lack of effect on pruritus in their model, I will briefly relate my clinical experiences with that drug. With the usual moderate dose of 4–600 mg/day on a material of 40 atopic dermatitis (AD) patients the therapeutic results were in general favourable, though when compared with a reference group on the antihistamine dimethinden, the latter was found more beneficial (Table II). On the other hand, Cimetidine® has multiple and more and less serious side effects, according to a compilation of the literature (see Table I). In addition, cerebral toxicity was recently stressed even for children (2). The conclusion is that Cimetidine does not seem to have an important part to play in the antipruritic therapy of AD.

The theoretical basis for PUVA in AD is similar to that of psoriasis, i.e. a favourable effect of UV can be expected on the skin condition in most cases. It is, however, a more difficult problem to understand its mechanism (Table III). As regards the clinical results, although several workers use this method for AD, only one large material is mentioned (1) where favourable results were registered after a relatively long period of time (on average, 36 treatments) and maintenance therapy was also needed.

In my experience, in a very small but thoroughly followed group of AD patients, PUVA therapy was efficacious in some cases, especially against ecze-

Table I. *Side effects of Cimetidine*

Leucopenia/bone marrow toxicity
Headache/dizziness/bradycardia
Gastralgia/diarrhoea/vomitus
Mental confusion (mostly in elderly who were ill and had renal impairment, or due to overdose)
Fever
Stevens-Johnson syndrome
Gynecomastia in men/male sexual dysfunction (due to hyperprolactaemia—as galactorhea in woman observed—or to increased gonadotropins or to antiandrogenic effect?)
Interference with: anticoagulants
Interference with: parathyroid hormone secretion
Interference with: insulin release
Transient increase in alkaline phosphatase

Table II. *Clinical impressions of the effect of cimetidine® on itch in cases of severe atopic dermatitis*

	No. of cases
I. Pilot study ($N=20$)	
Good effect	4
Moderate effect	4
No effect	12
II. Single-blind ($N=20$)	
Cimetidine better than usual antihistamine	3
Cimetidine equal to usual antihistamine	6
Usual antihistamine better than cimetidine	11
Ages of patients: 18–40 (28 women, 12 men)	
Dose given I: 600 mg/day; II: 400 mg/day	
Other treatment given: hydrocortisone/indifferent cream	

Table III. *On effectivity of Puva treatment*

	Empirically	Possible mechanisms
In psoriasis		
Sunlight	Mostly beneficial	Inhibition of DNA synthesis by keratinocytes(+effect on dermal structures?)
Puva		
In atopic dermatitis		
Sunlight, IR	Deterioration	Increased sweating → increased itch?
Sunlight, UV (B+A)	Improvement	Desquamation removing pore closure of sweat duct ostium? (But subsequent thickening of corneum may promote plug formation at the ostium.) By promoting blood flow—increased absorption of dermal infiltrates? Antipruritic? (But initially reducing itch threshold)
Puva	Improvement	

Table IV. *Effect of Puva treatment in cases of extensive and lichenified lesions of atopic dermatitis*

Evaluation according to a score system.
Maintenance doses: every week – every 2 weeks

Mean total dose	Approx. clearing of lesions: Eczematous (%)	Approx. clearing of lesions: Lichenified (%)	Pigmentation at last treatment
50 J/cm²	50	25–50	Moderately brown
50–80 J/cm²	50–80	50	Moderately brown
>80 J/cm²	80	50–60	Moderately brown
After Puva therapy (in autumn–winter–spring)			
Maintenance given	30–50	25–50	
Without maintenance	0–25	0–25	

$N = 8$. Skin types: II and III.

matous or prurigo lesions but less so against lichenification. Maintenance therapy was absolutely necessary to combat recurrences (see Table IV). Thus, similarly to Morrison (1), I consider that PUVA may be a favourable therapy for the treatment of AD but an intense and prolonged effort is necessary to achieve these results. Consequently, strict indications should be considered. Among the contra-indications it may be debated whether children with AD should be treated by this method, but I feel one should wait with these cases until we have a clearer picture of possible long-term side effects. In addition, even the relative contra-indications should be considered and especially stressed that lubrication must be given against the drying effect and itching caused by PUVA therapy (Table V).

Table V. *Puva therapy for atopic dermatitis*

Indications:

Selected cases with particular resistance to conventional treatments and with extensive lesions, especially adults
Selected cases with particular resistance to conventional treatments and with extensive lesions, especially adults

Contra-indications:

Mild types of atopic dermatitis
Intolerance to light/taking of photoactive drugs
Eye disease
Liver/renal and other diseases.
Pregnncy
Childhood (?)

Relative contra-indications/special considerations:

Severely ichthyotic or extremely dry skin
Very low threshold of itch
Recent pyococcal superinfection

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DISCUSSION

Soter (Boston). Q: When you administered Cimetidine, did you give it in divided doses and did you study delayed-type sensitivity reactions?

A: I gave divided doses and perhaps the doses given were not too high. I did not conduct delayed studies.

Q: Were the cancerogenic effects of PUVA considered?

A: Yes, we were very cautious and gave it only in "desperate" cases and never to children. I think PUVA cannot be used as a standard method in the therapy of atopic dermatitis.

Dobson (Buffalo): The main concern is that PUVA is capable of producing skin cancer developing from normal skin within a few years. If it is used only in the short term, as indicated, then it is impractical in a chronic disease.

Goldberg (Palm Beach): Most of the centers I contacted in the US do not use PUVA for atopic dermatitis. Perhaps we may use it to some very limited extent, but it should be remembered that it requires about 2 or 3 times the amount of treatment given for psoriatics.

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THE USES OF PUVA IN ATOPIC DERMATITIS

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Abstract. PUVA is an acronym which stands for the use of Psoralen as a photo-sensitizer for patients exposed to ultraviolet light type A (320–400 nm). Such use was brought to worldwide attention by Parrish et al. in 1974. Its deserved popularity is warranted by a remarkable improvement in over 85% of psoriatic patients. It was expected that PUVA would be tried for many skin conditions. Good results have also been claimed in atopic dermatitis (AD), mycosis fungoides, alopecia areata, prurigo, parapsoriasis, and urticaria pigmentosa. This paper represents a compilation of the reports from several PUVA treatment centers in the USA concerning AD. The reason for the improvement in these skin conditions which vary considerably in their etiology and histo-pathology, remains to be explained.

Key words: PUVA therapy; PUVA treatment centers

Patients with atopic dermatitis (AD) were treated in a manner similar to that commonly used for the treatment of psoriasis namely, 0.6 mg/kg bodyweight of 8 methoxypsoralen was administered orally approximately 2 hour prior to exposure of the patient to UVA 2–3 times per week up to the tolerance of the patient, i.e. just less than that amount which produced a feeling of and/or a visible redness of the skin plus itching. This amount was gradually increased, depending on the type of skin of the patient until 5 and up to 10 joules/cm^2 of UVA was given. This range was usually sufficient to produce a gradual improvement of the affected skin. Laboratory studies of the complete blood count and the blood chemistry including the anti-nuclear antibody test were performed to establish a base for further studies as the treatments proceeded. Eye studies were also taken and monitored.

Although my primary interest is in psoriasis, having a complete PUVA set-up has made it possible for me to test its value for other disorders. Although less common diseases such as mycosis fungoides, parapsoriasis, and urticaria pigmentosa are reported as being responsive to PUVA, I have been more interested in using it for the treatment of AD, vitiligo, and alopecia areata. This use has been somewhat limited by the fact that patients under the age of 12 are excluded from PUVA treatment in USA. Personal communications have been used to evaluate and broaden the results of my own experiences. Atopy was a term coined by Coca (1) in 1923 a has been applied to dermatitis (atopic) and other disorders. A review article on this subject by Hanifin & Lobitz (2) emphasizes the confusion and difficulty in establishing the diagnosis. Speculations on the pathogenesis vary considerably. This makes an explanation of the possible improvement in AD by PUVA even more difficult, since the histo-pathologic findings of AD and psoriasis are so different. This is more so where we find further differences in the pathogenesis of mycosis fungoides and urticaria pigmentosa. The most extensive study of the use of PUVA in AD is that of Morison et al. (3). These authors described the clearing of atopic eczema in 15 patients with PUVA therapy. The authors have allowed me to show typical slides of some of their patients and also sent me a follow-up note on their patients after 4 years of treatment (4). The notes that especially interested me were: that P-UVB did not have the beneficial effects that P-UVA had; the beneficial effects that PUVA has on blepharitis; the fact that the amount of PUVA needed to clear atopic eczema was about twice that needed for the clearing of psoriasis, and the unfortunate fact that 20% of these patients developed herpes simplex.

The end results of this treatment by Morison et al. (3) as of February 26, 1979 are:

one patient who had an early spontaneous remission is still clear;

one patient who cleared dropped out of treatment;

one patient needed two treatments/week for maintenance (this was considered an excessive amount of treatment for this disorder);

two patients are on weekly treatments, but still have 20% involvement;

ten patients are on PUVA and are controlled with

requirements of weekly (5 patients), every 2 weeks (3 patients) and monthly (2 patients) treatments.

Personal communications regarding PUVA therapy for AD in several other PUVA centers are as follows:

Mount Sinai Medical Center (5), Miami Beach, Florida: Dr Phillip Frost states he has had no experience in the use of PUVA for AD.

University of Michigan Medical School (6), Ann Arbor, Michigan: Dr John J. Voorhees states he does not treat AD with PUVA because of the possibility of serious side effects, since AD requires more treatment time than psoriasis. This is exemplified in the article by Tam et al. (7) which describes the occurrence of Bowen's disease and squamous cell carcinoma in a patient age 32 who had a most unusually high cumulative dosage of UVA, namely 3 700 joules/cm^2 as part of his PUVA therapy.

Finally a note for those physicians to whom it has not become available because of cost. Petrozzi & Kligman (8) describe PUVA without specialized equipment in the Arch Dermatol *114:* 387–390, 1978.

It may be that AD in Norway is more of a problem than in many areas of the USA, and therefore PUVA treatment may be important to have available. AD patients in my own practice have been limited, but in general my results have agreed with those of the Harvard Medical School group.

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EXPERIMENTAL TREATMENT IN ATOPIC DERMATITIS: IMMUNOLOGICAL BACKGROUND AND PRELIMINARY RESULTS

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Abstract. Experimental treatment in atopic dermatitis was undertaken with transfer factor, hyposensitization, or topical sodium chromoglycate. Both transfer factor and hyposensitization in open trials produced some clinical benefit. In both cases all patients could be controlled with medium strength topical steroids during therapy. In the latter case consumption of topical steroid was measured and found to decrease. Improvement generally followed a decrease in serum IgE after an initial rise. No significant changes in T-lymphocytes or serum IgE were observed during transfer factor therapy. Topically applied sodium chromoglycate 10% in white soft paraffin in a double-blind trial was no better than the placebo.

Key words: Hyposensitization; Transfer factor; Topical sodium cromoglycate

Management of severe atopic dermatitis (AD) is a great challenge to dermatology. As stated by Rajka (13) almost every conceivable internal remedy, which has been used anywhere in the world for recalcitrant skin diseases has been tried in AD, but only a few drugs have stood the test of time. The data we wish to present here are, as stated in the title, both experimental and preliminary. They do not seem to solve the overall problems of treatment in AD, but may, however, add somewhat to our understanding of it. We report here on three trials, one with Transfer Factor (TF), one with hyposensitization (HS), and one with topical sodium chromoglycate (SCG).

TRANSFER FACTOR

Patients with severe AD have in general a pronounced increase of IgE in their serum (4, 8). They also often show signs of a decrease in cell-mediated immune reactivity, such as decreased skin reactivity, low numbers of circulating T-lymphocytes in peripheral blood, a decrease of PHA reactivity of lymphocytes, and immunosuppressive factors in serum (3, 11, 15). Also, it has recently been shown that atopics seem to have an impaired monocyte function (9). Most of these findings fit well within the framework of Szentivanyi's beta adrenergic theory of the atopic abnormality (15).

The lack of immunological balance may be a major factor in the occurrence of atopic symptoms. The decrease in cell-mediated immune reactivity and its possible significance for the occurrence of symptoms has led to the use of immune-stimulation therapy in patients with atopic dermatitis. Our own results with TF (10) in mycosis fungoides, where patients during therapy showed an increase in the number of circulating T-lymphocytes and a decrease in serum IgE, together with two promising case reports from 1975 (1, 14), where TF improved clinical symptoms in AD, led us to try this therapeutical approach in 3 adult patients. All 3 had decreased numbers of T-lymphocytes in peripheral blood, no release of migration inhibitory factor (MIF) after PPD-stimulation, immunosuppressive factors for DNA synthesis in serum, and high levels of IgE.

The effect of TF was evaluated clinically and *in vitro*. There was an improvement in the patients' disease in that there were no admissions during the treatment period of 1½ years, compared with tree, two and one admission during the preceding 1½ years. Also, during the last 12 months of treatment, none of the patients had secondary bacterial infections, and their use of topical steroids could be limited to a medium-strength preparation (Locoid®). It should be pointed out, however, that none of the patients ceased to have clinically pronounced AD. Fig. 1 gives an indication of the number of T-lymphocytes in blood. The number was low from the beginning, but continued to be so throughout the treatment. In general, the variations observed were similar to the variations found in healthy donors. Fig. 2 shows the patients' *in vitro* reactivity to PPD in a leukocyte migration test. Most of the migration indices were

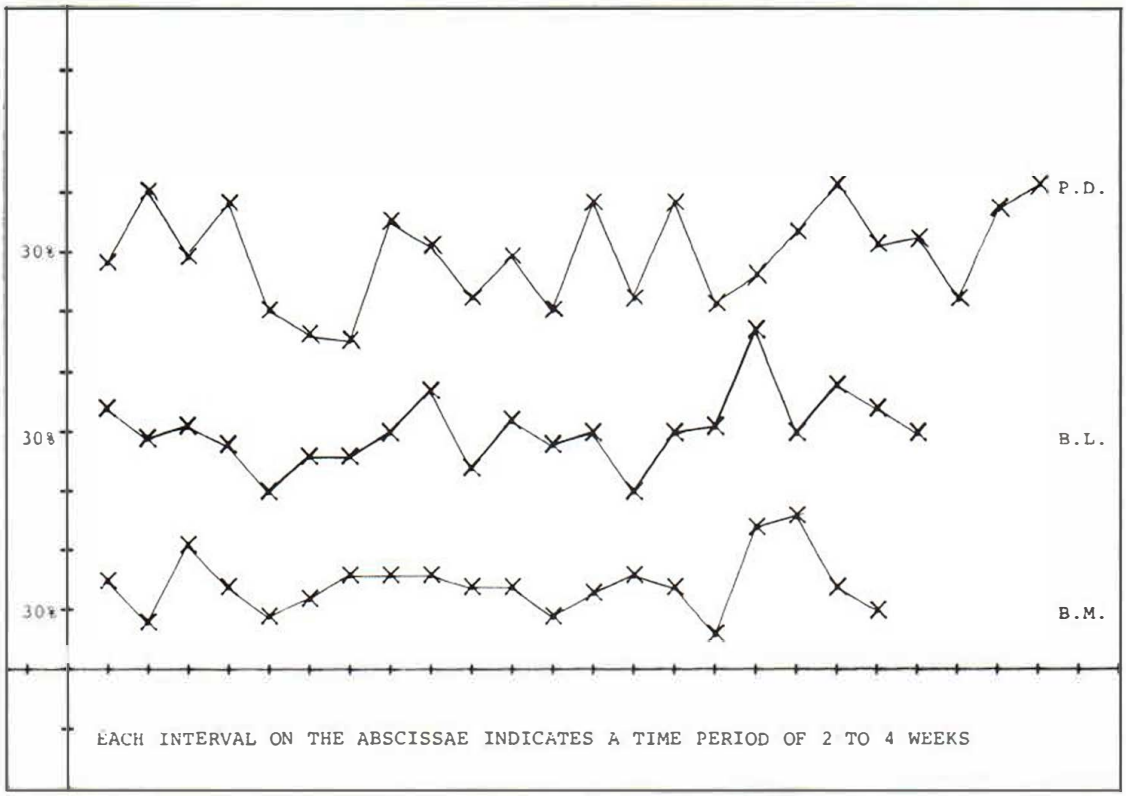

Fig. 1. E-rosette forming lymphocytes in patients with atopic dermatitis during treatment with transfer factor.

found in the upper part of the normal range, indicating only slight inhibition. It should be noted, however, that low values appeared early during treatment. This could be an effect of TF.

Immunosuppressive factors in serum were sought by adding patient's serum to lymphocyte cultures from two healthy donors and stimulating the lymphocytes with PHA. In one patient we found immunosuppressive factors during the first 6 months of treatment (Fig. 3). In another patient immunosuppressive factors were found on one occasion early during treatment.

Fig. 4 shows the variation in IgE levels in the patients' sera. Again we found variations, but none related to treatment.

The only double-blind trial so far (7) did not show

Table 1. *Clinical response following hyposensitization together with responses in serum IgE*

Pat. no.	Age/sex	Length of treatment (months)	IgE (units)			Clinical response
			Before	Highest	Latest	
1	32/F	24	3 680	4 480	2 490	Improved
2	32/F	24	6 370	7 500	3 540	Improved
3	58/F	20	11 300	22 900	3 970	Improved
4	23/M	18	4 418	9 450	6 570	Improved
5	31/F	22	2 550	7 200	2 730	Slightly improved
6	45/F	26	1 790	3 110	2 930	Slightly improved
7	33/F	24	1 580	1 860	1 510	Slightly improved
8	25/F	30	4 930	5 010	4 890	Unchanged
9	33/M	15	4 261	10 400	6 540	Worse

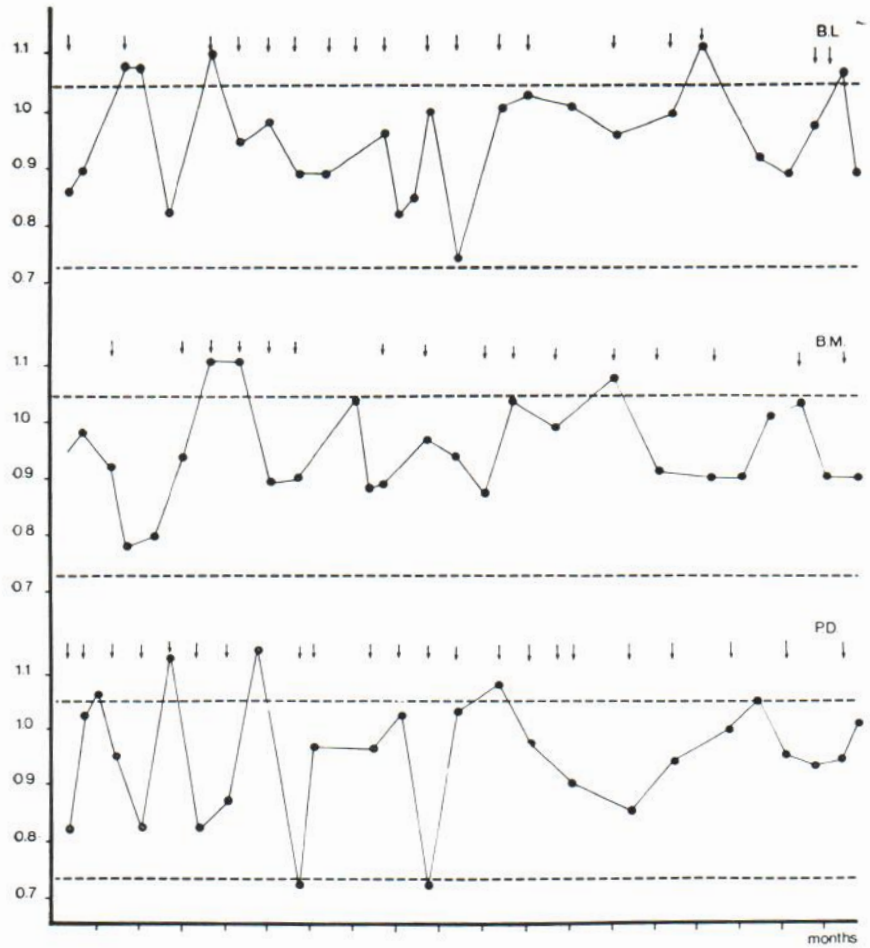

Fig. 2. Variations in cellular immunity to PPD as determined in vitro by the leukocyte migration test. The results are expressed as ratios between the migration areas in cultures with AB serum and PPD, and with AB serum alone. The range for normal persons is indicated as the average migration index (0.88)$\pm 2 \times$ S.D. Arrows indicate the times of transfer factor injections.

any beneficial clinical effect. We have recently started a double-blind trial of TF in 12 adult patients with AD. The dosage is higher than used by others and increased in comparison with our first open study. The dosage being 2 units, equivalent to extracts from 1×10^9 leukocytes every other week. The patients will be treated for 1 year. Among our initial laboratory findings, which included studies on subpopulations of T-lymphocytes in altogether 16 patients, we found a slight reduction of T-lymphocytes with F_c-receptors for IgG (Fig. 5), whereas the subpopulation with receptors for IgM was found to be slightly increased (Fig. 6). Findings during therapy are not yet available.

Table II. *Urinary histamine per 24 hours in 7 atopics prior to and following 6 months of hyposensitization*

	Urinary histamine (μg/24 h)	
	Before	After
4	37	
4	37	37
6	156	63
7	75	60
8	34	118
9	47	120
10	28	69
11	51	28
Average$\pm$S.D.	61$\pm$45	71$\pm$36

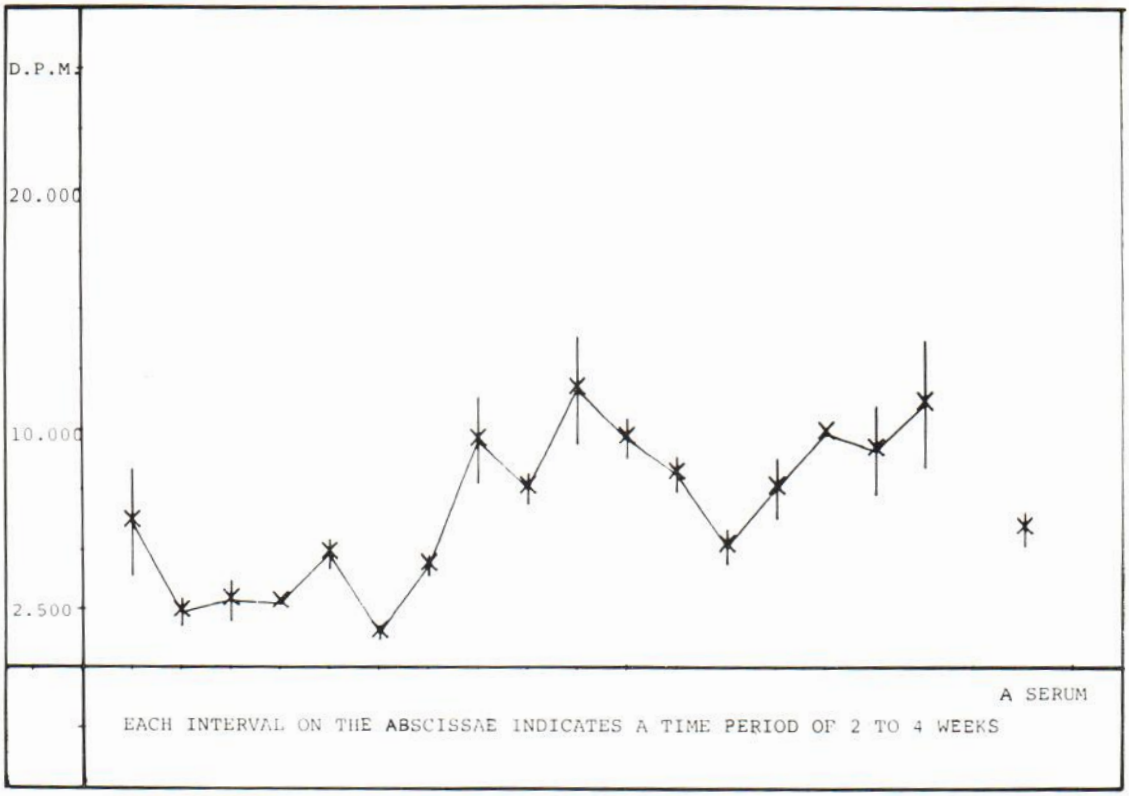

Fig. 3. PHA reactivity of lymphocytes from one normal donor after addition of 10% serum, measured by sub-optimal PHA concentrations in cultures.

SPECIFIC THERAPY

How hyposensitization works in respiratory atopics is not fully understood, but it is a common finding that following an initial increase in serum IgE, this parameter later decreases (2, 5, 13). The concept of an effect of blocking antigens which act against reagins (IgE) is still attractive to many allergologists, while others suggest that hyposensitization acts rather by a stimulation of specific suppressor T-lymphocytes (5).

Table III. *Average consumption of Locoid® cream during hyposensitization, in 6-month periods*

	Period no.			
	1	2	3	4
No. of patients	9	9	9	7
Consumption (g)	355	205	95	103

Due to conflicting data on the results of hyposensitization (13) in AD, we felt it reasonable to restudy this approach in patients with severe disease, using an allergen which is constantly found in the sensitized patients' environment.

Nine patients sensitive to housedust mites were hyposensitized. Injections were given in increasing dosages with intervals increased from 1 to 8 weeks. All patients were allowed to use a medium-strength topical steroid (Locoid®) according to their needs. The cream or ointment was delivered by us and the amounts used were measured.

Four of 9 patients improved (Table I), 3 patients showd slight improvement, one was unchanged, while one patient—the patient treated for the shortest period—was found worse. Most (but not all) patients followed the pattern of an increase in serum IgE, followed by a decrease. Urinary histamine (Table II) was studied in 7 patients (12) and was found unchanged following 6 months of treatment and generally to be within normal values. One

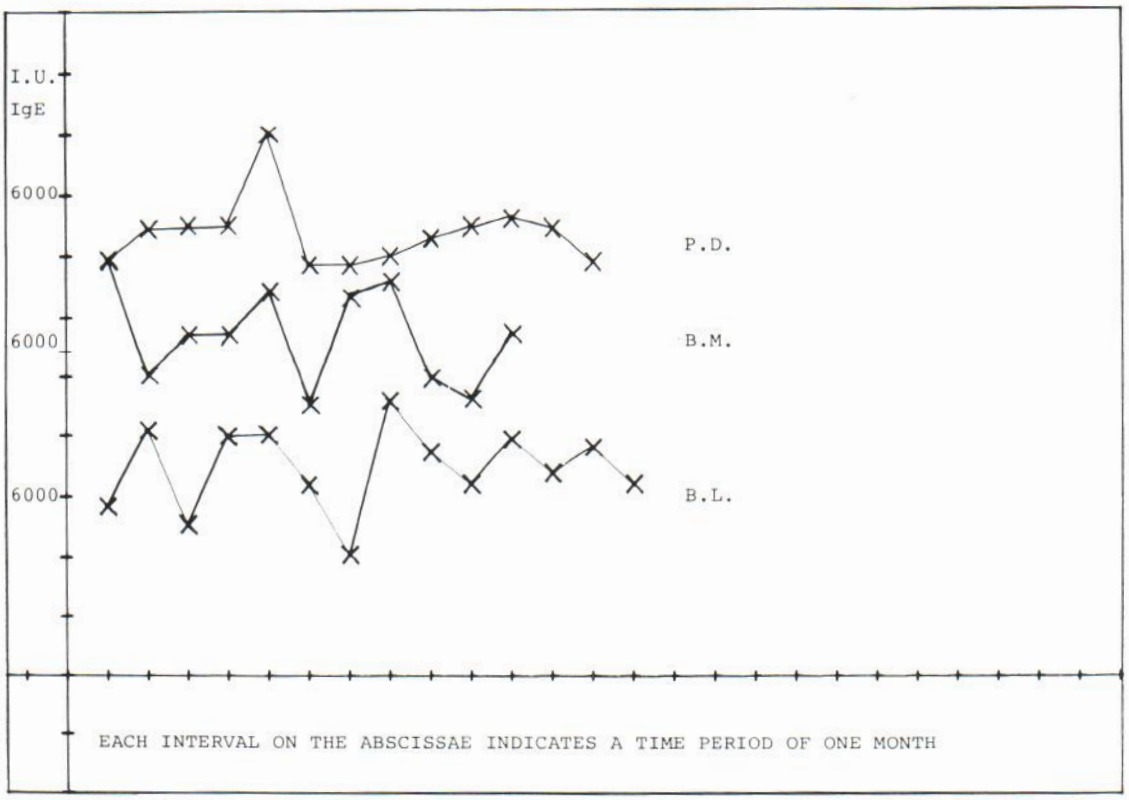

Fig. 4. IgE in serum during transfer factor treatment in patients with atopic dermatitis.

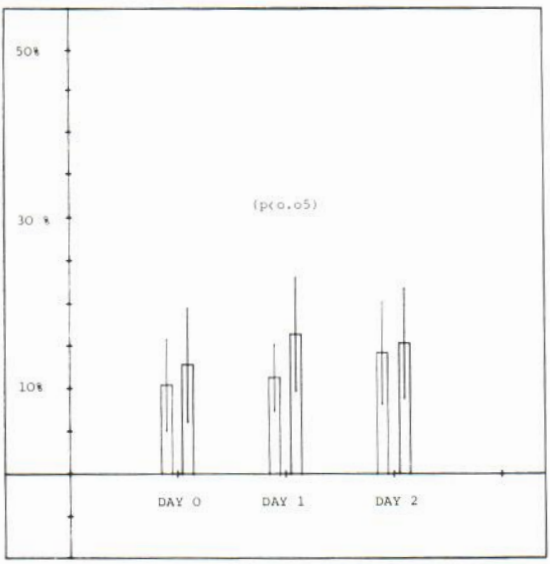

Fig. 5. Tγ-lymphocytes in peripheral blood from patients with atopic dermatitis and from normal donors.

parameter, however, indicated a more general improvement; the consumption of topical steroids (Table III) was reduced during treatment. It is our present opinion that it is worthwhile to proceed with investigations into the effect of hyposensitization in severe AD. This is especially so because we have today better allergen extracts and better tools with which to monitor the immunological status of the patients.

SODIUM CHROMOGLYCATE

Sodium chromoglycate (SCG) was first introduced as a prophylactic agent in the long-term management of asthma. The most important mode of action is believed to be related to its ability to inhibit mast cell degranulation and mediator release. The beneficial clinical effect found in allergic asthma,

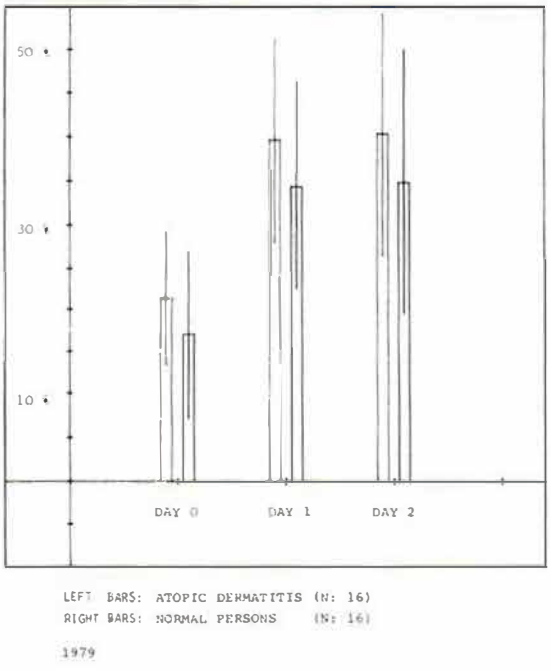

Fig. 6. Tμ-lymphocytes in peripheral blood from patients with atopic dermatitis and from normal donors.

allergic rhinitis, and allergic conjunctivitis has suggested a trial also in AD, in spite of the different target organ and the less well known pathogenesis. A preliminary double-blind trial was carried out by Haider (5) and suggested an effect. We have reported (17) on another, more recent trial on topically applied SCG.

A total of 35 children with AD, aged 2½–15 years were entered into the study. Almost all patients were using a topical steroid at the time of trial entry. 17 were on a double-blind basis allocated the active preparation 10% SCG in soft white paraffin, while 18 received placebo. The ointment was applied twice daily.

The clinical evaluation consisted of assessments of redness, dryness, lichenification, cracking, hyperkeratoses, excoriation and scaling graded from 0 to 3. Diary cards included the patient's or parents' evaluation of itch, sleep, and severity of condition.

Analyses of diary cards, weekly score totals, and of assessments, revealed only a single significant difference favouring the active preparation: fewer excoriations were found on the lower limbs at week 1. Nine patients in the placebo group and 7 in the SCG-treated group dropped out of the study before completion, the chief reason being worsening of the skin symptoms.

It was our conclusion, that in contrast to the work of Haider, we were not successful in controlling our patients with topically applied SCG, and in our hands the active drug was no better than the placebo. The lack of effect could have been due to a too low dosage, poor penetration into the skin, or a lack of pharmacological effect on main pathogenic mechanisms.

COMMENTS

In conclusion, none of our three experimental trials has given us the answer to the problem of management of the patient with severe AD. We do feel, however, that none of the three approaches should be discarded at present. We find it necessary to continue to study attempts at IgE regulation in atopic dermatitis as well as trials with new pharmacological agents.

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DISCUSSION

Bonifazi (Bari). Q: Did those patients who improved with house dust, hyposensitization also have respiratory allergy? I would also like to know your opinion about the possible prophylactic significance of this type of treatment in children with AD having a family history of respiratory allergy with housedust antibodies—that is, that we are afraid will develop into respiratory allergy.

A: Respiratory allergy was not a major problem in these patients, but two of them had it. The major problem was extensive and very severe skin disease and we chose this group because we had an antigen which would be in the patients' environment all year round and was scarcely related to the seasons. About the future use in children, our allergens are better made today and many of the studies which were undertaken by dermatologists previously were done for too short a period, I would believe. I think one should use the experiments of the allergologists and proceed for up to three years before making an evaluation of the patients.

Vickers (Liverpool). Q: Do you think the reason why the chromoglycate is not effective in most of the trials is that we are not using it for long enough and it may be that we should go on for 2 to 3 months before abandoning what would be such an attractive drug locally?

A: We used it for 6 weeks and I can assure you that the patients and the parents found that that was at least 3 weeks too long. It would not have been possible to continue further.

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ATOPIC DERMATITIS AND SYSTEMIC TREATMENT WITH A NEW CHROMONE COMPOUND (FPL 57787) A DOUBLE BLIND CLINICAL TRIAL

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Abstract. In a double-blind cross-over trial, 23 adults with atopic dermatitis were treated systemically during two 6-week periods with a new anti-allergic chromone compound (FPL 57787) 18 mg four times a day or matched placebo in randomized order. Twenty patients completed the study: 11 preferred the active period, 9 preferred the placebo period. There were no statistically significant differences for any parameter. Four of the patients had drug-related dyspepsia. No laboratory side effects were noted.

Key-words: Atopic dermatitis: Chromone-carboxylic acid; Systemic treatment: Double-blind cross-over trial

In a recently published study (5) with a new chromone compound, FPL 57787, in the systemic treatment of adult atopic dermatitis (AD) we observed nearly identical recovery in the active group and the placebo group during the trial and there were no statistically significant differences in the clinicians' scores for any parameter. However, in the active group there was relatively little use of topically applied steroid. We therefore designed a new trial, using the active drug in a higher dose.

MATERIAL AND METHODS

The new drug is a chromone-2-carboxylic acid (FPL 57787) with the empirical formula $C_{17}H_{18}O_8$. The in vitro anti-allergic properties have been described earlier (5).

The material consisted of 23 patients suffering from AD. They all gave their informed consent after the trial had been fully explained. All were above 18 years of age and selected in accordance with the criteria laid down by Hanifin & Lobitz (4). Patients with severe AD and patients receiving systemic steroid therapy were excluded. Only women using effective contraceptives were accepted as participants. The patient characteristics are listed in Table 1.

The study was performed as a double-blind cross-over trial in order to compare the efficacy and safety of FPL 57787 with a matched placebo. After a 2-week baseline period, the two treatments, each lasting 6 weeks, were given in randomized order. The dose in the active period was 18 mg FPL 57787 four times a day. The study was carried out from October to December 1978. All previous treatment was stopped. After the baseline period the patients were seen once every 3 weeks. At each visit they were given 1 % hydrocortisone cream and asked to use topical treatment only when necessary. At the visits the clinician evaluated dryness lichenification, excoriation and dermatitis on a 0–3 scale and the extension in shading on the affected areas.

The following laboratory investigations were performed during the trial: ESR, whole-blood count, haematocrit, MCV, MCH, MCHC, differential white cell count, platelets, sodium, potassium, calcium, albumin, urea, creatinine, acid phosphatase, basic phosphatase, SGOT, SGPT, LDH, phosphate, urate, total lipid, bilirubin, cholesterol, iron and prothrombin time. Urine was analysed for blood, protein, and glucose. The statistical analysis for patients and clinicians' preference for one of the two treatment periods was made at the two-tail 5% level using a sign test for paired data.

RESULTS

The study was completed by 20 patients and both patients and the clinicians preferred the same treat-

Table 1. *Patient characteristics in an atopic dermatitis material of 23 cases treated with a chromone preparation (FPL 57787)*

Sex	Male	8
	Female	12
Age (years)	Mean	27.6
	Range	18–41
Age at onset (years)	Mean	1.5
	Range	0–10
Other allergic diseases	None	7
	Asthma	9
	Hay fever	12
Family history	None	4
	Eczema	11
	Asthma	8
	Hay fever	11
Severity of eczema	Mild	14
	Moderate	6
IgE (U/ml)	Mean	1 680
	Range	2–8 816

ment period. Eleven patients preferred the active period, while 9 patients preferred the placebo period. There were no statistically significant differences in the clinical assessments, in the patients' diary cards, or in the use of hydrocortisone cream. During the trial, 3 patients withdrew, 2 of them for reasons having no relation to the trial and 1 because of nausea, vomiting and dizziness starting in the active period. Furthermore, 3 of the patients who completed the study had drug-related dyspeptic symptoms in the active period. The laboratory tests were all within normal range.

DISCUSSION

In the field of dermatology, oral disodium chromoglycate (DSCG) seems effective in the treatment of food allergy, mastocytosis and dermatitis herpetiformis (1, 8, 7). Oral DSCG has also been used in AD, but only preliminary and uncontrolled investigations are available (6). There are also conflicting data about topically used DSCG in AD (3, 9).

The new oral anti-allergic drug, FPL 57787, has shown promising properties in in vitro investigations and in the systemic treatment of asthma (2). In patients with more atopic diseases or AD, which cannot be controlled by moderate use of topical ointment, the oral treatment by a non-steroid compound would be a welcome alternative in the management of AD.

Our first study (5) gave some evidence that FPL 57787 might be effective in the treatment of AD. However, we may conclude that the present trial could not demonstrate any effect of FPL 57787 in systemic treatment of AD. Furthermore, the applied dose resulted in some dyspeptic side effects.

ACKNOWLEDGEMENTS

Fisons Limited is thanked for supplying us with FLP 57787.

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DISCUSSION

Barnetson (Edinburgh). We have been carrying out a similar study on a similar chromone, double-blind, for 3 months on effective drugs and for 3 months on placebo and we have found that the drug had no improved effect over placebo. If food allergy plays any part in the atopic eczema, one feels that drugs like this should be having some effect, but it may be that one needs to study a chromone whose effect is localized to the gut.

Schoepf (Freiburg): Our impressions of this new chromone drug are a little bit better. We made the same double-blind study as you, with the same design, and of 11 patients 8 showed improvement and 3 no effect, but I think that we have to distinguish between several types of atopic dermatitis. I feel that the patients with chronic lichenified eczema did not respond and the other atopics with flushes and acute deterioriations responded.

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CONCLUSIONS

Dr Nicholas A. Soter

I like to thank Prof. Rajka for this opportunity to survey the papers on the clinical and pathophysiological aspects mainly presented on the first day and must confess I undertake this task with humility and trepidation. Perhaps you will permit me to divide this consideration into four main areas for the purposes of discussion. Firstly, I would like to consider the diagnostic criteria because they are incredibly important—perhaps *the* most important, because we must know what we are studying. These were considered by John Hanifin and Prof. Rajka, who pointed out that there are no biochemical markers for the diagnosis of atopic eczema, either biochemical or genetic. After a lively discussion I think it was agreed that there are certain major clinical features that are important. These really consist of a very prominent pruritus and secondly of a distinctive clinical morphology and distribution pattern. Thirdly, a chronic relapsing course and finally a personal or family history of other manifestations of so-called atopic diathesis. Other less frequently associated features were certainly considered, but I think these four are the major manifestations.

Next, Meenan delightfully discussed Morgan's fold, which is the infraorbital fold in atopic individuals. It seemed clear that this is perhaps an important feature in children under the age of 4, whereas after the age of 4 in older children, adolescents and in adults, its importance is less clear and it seemed evident to me that the natural history of this—or what happens to this fold—is really unknown, as we heard that it disappears and then we heard that it is present in adults later. I would like to echo this plea, however, for the maintenance of clinical skills when faced with the clutter of laboratory and scientific studies we are being deluged with now and in the future.

The second area I would like to discuss is my own work on the histopathologic alterations in atopic eczema. Mainly this provided a newer morphologic technique, using 1 μm thick sections which have shown differences in acute (vesicular) and chronic (lichenified) lesions in atopic skin as well as abnormalities in supposedly normal skin. Furthermore, although changes in venules were found in older studies, the microvasculature has never been adequately discussed and elucidated. Moreover, there are differences in infiltrating cell types and finally there was the recognition that in chronic lesions there was nerve damage which appeared to be present in clinically involved skin as well. Whether these vascular changes suggest that there is an underlying vascular abnormality in the skin of individuals with atopic dermatitis or whether it merely reflects previously involved sites is unclear. But I think it is a start. I would like to also suggest that this technique may be used to study other clinically similar eczematous dermatitides.

The third area that we should mention I will call 'studies on physiologic manifestations'. Dr Aly began with a review of the biological flora present in the skin of atopic patients and it was rather intriguing that there were increased numbers of staphylococci albeit many of them are resistant to penicillin therapy and very intriguingly an absence of lipophilic diphtheroidal organisms. This would suggest studies both in vivo and in vitro of the interrelationship of these two organisms and perhaps one ought to investigate both acute and chronic lesions. In addition Dr Aly mentioned preliminary studies on differences in adherence of bacteria to epidermal cells from atopic individuals. Certainly these ought to be pursued. Secondly, Dr Dobson reviewed the literature on sweating in atopic individuals and it appears that the rate of sweating as well as its composition are normal. He noted the substances that had been defined in sweat. They are enzymes, ABO blood group substances, albumen and certain immunoglobulins, with the presence of IgE still to be clarified. It was important I think that at least in the eccrine sweat gland there was no beta-blockade as there is in other tissues. And finally he considered the role of pore occlusion, suggesting that if it is important at all it might be so only in a minority of individuals.

Thirdly, Dr Thune reviewed the problems of blood

vessel reactivity in atopic individuals with the consideration of both white dermographism and the delayed blanch. The skin was studied using a reflectometer which measures colour and a pulse meter which would measure vasal constriction or dilatation. These studies were performed after various types of chemical experimental challenge. If I remember his data correctly, I think we can say that some patients have responses that are no different from those of normal individuals, some people have abnormal responses consisting of either vaso-constriction in certain instances, in vaso-dilatation in others. So we have the whole spectrum of vessel response. And in some patients these responses even fluctuate. I think that this variety of response suggests that these studies ought to be extended and ought to involve time course investigations in individual patients.

Fourthly, Dr Aoki studied nocturnal scratching in atopic individuals, although I was very intrigued by the fact that we all scratch quite a lot when asleep. Much of this scratching occurred in what he called a cluster pattern and there appeared to be no relation to serum IgE levels. He suggested that these studies will continue, looking at the depth of sleep to determine whether or not there were any alterations there. Next, Prof. Rajka studied pruritus in patients with atopic disease that was limited to skin—or, as he called them, a pure atopic population. And his stimulus was the application of trypsin to the skin. He showed us that the response appeared to be greater in atopic dermatitis patients who had the highest level of IgE, which suggested to him that this response was more severe in patients with more extensive disease, since IgE levels certainly reflect more extensive disease. Finally, in experimental studies on pruritus performed by Dr Harnack, it was suggested that atopic patients experience a longer interval of pruritus than other individuals, usually greater than 2 minutes. However, he was cautious and said that this certainly was not a diagnostic feature of these patients.

The last area, perhaps the most intriguing and the most complex area, is what I would like to call 'bad chemical probes in atopic patients'. This mainly revolves around studies on cyclic nucleotide metabolism, not only in whole tissues, but also in isolated cells. When evaluating these studies it is often difficult to compare results from different laboratories and one must bear in mind that at doses of stimulating agents present when very large doses are used, such as 10^{1} or 10^{2} molar, most people in the field would be sceptical of the results—really one wants to get into the range of 10^{-5} or so molar for eliciting agents. A very important consideration on evaluating these studies is the purity of the cell populations. Finally, when one evaluates these studies I think it is fair to say that often the differences are due to technological and methodological techniques rather than the underlying biology being so conflicting. So consider these three areas when you evaluate the literature. This section was started by Dr Szentivanyi who reviewed his beta blockade theory of atopy, which consists basically of diminished in vitro responses of cells and tissues to beta adrenergic agents such as the catechols. The observations were made originally in patients with asthma, but they have subsequently been extended by various studies to individuals with atopic eczema. With appropriate stimulation in atopic tissues there is a diminished response in cyclic AMP with normal responses to cyclic GMP. This decrease in cyclic A to cyclic G ratios suggests that, in the presence of normal phosphodiesterase metabolism, early events are operative in this abnormality. Furthermore, he showed in lung tissues and circulating lymphocytes that there is an increase in alpha receptors, in contrast to the normal predominance of beta over alpha receptors. Intriguingly, treatment with hydrocortisone restored this abnormal ratio to the usual 5:1 beta/alpha predominance. Perhaps such studies will allow the differentiation of basic atopic abnormalities from drug-induced changes in airways and even the skin. I think there was a note that the aforementioned abnormalities may be present in inactive as well as active disease and the stimulus to activity in patients is really unknown. It was suggested that infection—and especially viral infections—may be one trigger for activation. Moreover, the mention of the interconversion of alpha and beta receptors was mentioned, although few studies are available as yet. I think they are going to be incredibly important in the future and should be considered in any future studies.

Dr Szentivanyi's presentation was followed by one by Dr Giannetti who studied beta-adrenergic blockade in circulating lymphocytes from patients with atopic eczema. He showed inhibition of proliferation by PHA stimulation by isoproterenol. The presence of such inhibition in patients with either mild or severe disease was noteworthy—there seemed to be little or no difference and, furthermore, in

patients with any combination of the atopic diathesis, i.e. in skin, with or without respiratory disease.

Dr Maubeuge and colleagues studied receptors on lymphocytes from atopic patients with skin disease, usually with respiratory disease as well, using a newer radiolabelled substance that preferentially binds to beta-receptors. She showed that the number of beta-receptors was the same, though the binding affinity was quite different, suggesting that there are qualitative differences in atopic individuals when compared with non-atopic individuals.

Finally, Drs Marcelo and Voorhees reviewed their studies on cyclic nucleotide responses in epidermal cell cultures in vitro from both mouse and human skin (I couldn't imagine skinning 50–60 mice), but at any rate in humans low inputs of beta-adrenergic agents appeared to stimulate and high doses of beta-adrenergics appeared to inhibit cell proliferation. This model may be applied in the future, I think, to studies on the epidermis of patients—perhaps those with atopic eczema. It might be a fruitful method not only for studying lesions at different ages but also to study pharmacologic modulation in the system.

To sum up, then, I think we can say that although there is no biochemical marker in patients with atopic eczema, it is obvious that reasonable criteria can be applied in its diagnosis. Newer laboratory techniques can be used to study both histopathology and biochemical abnormalities and these biochemical probes may not only permit elucidation of underlying pathophysiological alterations, but also point to advances in therapy in which controlled clinical trials are requisite and mandatory.

Dr Jon M. Hanifin

Well, before I start I'd like to express my sincere gratitude and pleasure to Prof. Rajka for organizing this meeting. I don't think anyone else could have done it. The obstacles and the difficulties make me tired, just to think of them. For all those of us who spend our time struggling with atopic dermatitis, this gathering is absolutely essential. We're a very small family and the elephant is very large. So to you, Professor Rajka, I say thank you very, very much.

My task now is to discuss the immunologic and allergic features that were presented here. Obviously there are so many that I can't do justice to all of them. I'll start with the presentations on food inhalant and contact allergy. I think we can summarize by saying that these types of allergy do exist, but the frequency with which they play a role in atopic dermatitis is uncertain. I think it depends greatly on who is feeding the 'elephant', how many times food actually plays an etiologic role. But in my own experience I think that the combination of food, inhalant and contact allergy triggering atopic dermatitis is probably in the realm of 10% of all the patients that I see. So it definitely happens; we have to be very aware of it; I look very closely for this type of trigger, but I don't think it's the whole causation. I think that when we're talking in terms of food allergy it is quite obvious that RAST skin tests and clinical signs may be helpful in our elucidating this factor. Food allergy has to be confirmed by challenge and it has to be eliminated for prevention. I think that Dr Atherton's very ambitious studies are essential to our eventual understanding of the value of prevention in food allergy. It is difficult and actually impossible to clearly separate immunologic studies from most of the other laboratory investigations that we have heard about these past three days. In reality the final resolution of the immunologic peculiarities of atopic dermatitis will be explained in biochemical terms. I think the sooner we get to biochemistry, the better off we shall be. But at this point there is obviously great confusion. All of the immune function tests are really only reflections of an underlying biochemical disruption. But they are essential tests so that we can, when the biochemical assays become available, study the functional tests.

We have all spent time here talking and thinking about whether immunologic defects are primary or secondary to the dermatitic inflammation or infection. The Strannegårds have struck a blow to the latter idea of being a secondary effect, by their finding of decreased T cell numbers in the blood of 1-month-old infants of atopic parents. This certainly suggests a genetically determined defect and they suggest the possibility of this defect being in the thymus or in thymus-influenced cells. Obviously follow-up and diagnostic interpretations are still pending in this study, but it is very exciting and I feel that one of the recurring themes in this symposium is the need for longitudinal studies. Now the Strannegård's studies contrast with those of Dr Uehara in a sense, because as he has so beautifully done in the past, he has again reminded us that immune deficiency phenomena—maybe not all of

them, but at least some of them—may be transient and they may be seen in non-atopic dermatitis. For me, a plain message of his work is to watch the composition of our control group, be sure that we are looking at non-atopic dermatitis in our studies. Dr Aas too has made the plea for us to consider sub-populations. It is very gratifying that in so many of these studies the investigators took into account various factors such as age of onset, presence or absence of eosinophilia, presence/absence of increased serum IgE, the presence of pure atopic dermatitis as opposed to atopic dermatitis combined with allergic respiratory disease. I think that in the future we must consider these possibilities that there are sub-populations of patients and that we may find our differences defined more clearly in these sub-populations.

Dr Byrom has done a beautiful job of reviewing the controversial areas of rosette-forming T cells in atopic dermatitis. He has shown us that E-rosette-forming cells are clearly below normal levels but that unknown factors in fetal calf serum and in crude thymic extracts will correct the rosetting deficiency and his findings support the idea of an immature lymphocyte sub-population in this disease.

We continue to have the lymphocyte transformation controversy. The Strannegårds have for example noticed decreased LIF production. The lymphocyte transformation question has ping-ponged back and forth as long as we have been studying it. It is a good reminder that we all use different techniques, we use different concentrations of mitogens, we use different kinds of culture, we are examining a patient at only one time in his course, one brief point in the disease. And I think for a clear interpretation of so much of this data we have to clearly define at what point in the disease course the patient is being evaluated. This was pointed out by Lobitz and colleagues in 1972, when they first started this. They pointed out to us that patients with very marked PHA depression at one point may have supernormal responses during clinical remission. And my own studies have merely confirmed this. Studies, in press, have also shown that PHA depression is reversible in vitro, hinting at short-lived suppressor cells, but we have been unable to find them. In fact, Dr Zachariae and his colleagues from Aarhus suggest just the opposite: that suppressor cells may actually be decreased, based on the results of T gamma marker technique. These are excellent studies and there is a great need for further characterization of sub-populations. The findings of the Aarhus group would be consistent with the theoretical considerations explaining high IgE production in atopic dermatitis and Dr Hovmark's studies and a recent report by Buckley suggest that, at least in some atopic dermatitis patients, the lack of suppressor control may be related to the unrestrained IgE production that we see in some patients. The entire realm of lymphocyte sub-populations is extremely uncertain at the present time. It has totally confused immunologists and so we cannot feel too badly that we too are confused. We need to consider sub-populations, but we also have to realize that the techniques are still limited—at present the markers are less than ideal, they require functional confirmation frequently by several different techniques and there may be several different populations of T suppressor cells.

For those of us who are drifting away from the purely immunological realm, Dr Saurat's studies are very interesting and increased whole-blood histamine release in atopics may help us to understand the pathologic findings in atopic dermatitis. One problem that continues to confuse those of us who are interested in histamine's role in atopic dermatitis is why atopics show increased histamine sensitivity in the skin and in certain lymphocyte function assays and yet they show the same blunted cyclic AMP response to histamine as they do to beta-adrenergic agents. It may be that, as Dr Szentivanyi suggests, it is necessary to examine these results carefully in the prospectives of receptors and receptor inter-conversion. I think that in spite of our many different interpretations of the immunologic events in atopic dermatitis, we need to look mostly for areas of agreement. There are defects of multiple tissue systems in this disease—the epidermis may be abnormal, in nerves, B lymphocytes, T lymphocytes, phagocytes, even mast cells. This is all suggestive that there is a common basic defect in atopic dermatitis and I think we should not be too depressed by the fact that we find slight differences due to technical variations.

The one area that has seen consistency by numerous workers and various laboratories is the blunted cyclic AMP response to adrenergic agents, at least, probably also to prostaglandin E and histamine. There is also general agreement on the increased responsiveness of these patients to cholinergic and alpha-adrenergic agents. So I think there is good agreement that there is a defect

somewhere hovering around this cyclic nucleotide system and all of these findings may be reflections of a common basic defect. Whether it is intrinsic to the cell, or whether it is caused by extrinsic factors remains to be shown. I think Dr Giannetti's studies have very nicely helped us to move into the realm of immuno-pharmacology, if one can use that term. I think that these studies may also lead us to this badly needed biochemical marker for the disease. So I believe we are much closer and although some of us have been frustrated at this meeting by the fact that we have not come up with any definite answers—we only have more questions—I think they are better questions and I think we all have new answers and new directions to follow in answering these many questions.

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POSTER AT THE INTERNATIONAL SYMPOSIUM ON ATOPIC DERMATITIS

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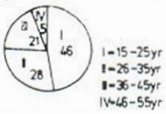
ALLERGIC REACTIVITY IN PERSISTENT ATOPIC DERMATITIS (KOROSSY, S., István Hospital, Budapest)

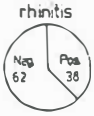
Sex distribution

Age distribution

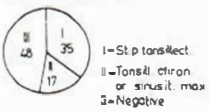


Familiar incidence

Associated bronchial asthma and/or atopic rhinitis

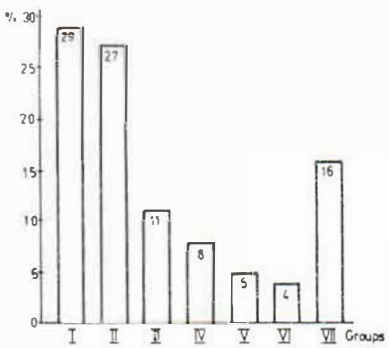


ENT infective foci



IMMEDIATE-TYPE REACTIVITY

Intracutaneous positivity



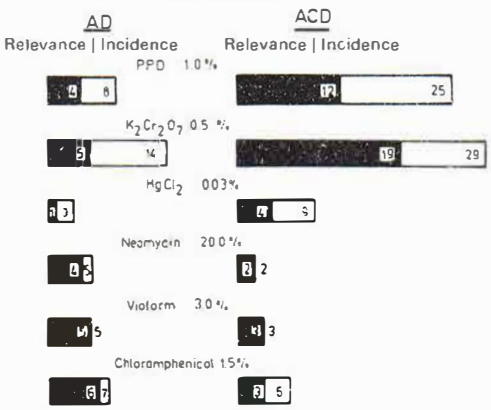
I = Inhalants (Bencard A1+A2)
II = Inhalants (Bencard A1)
III = Pollens (Bencard B2+B3)
IV = Mixed moulds (Bencard A13)
V = Inhalants (Bencard A2)
VI = Others
VII = Negative

DELAYED-TYPE REACTIVITY

Epicutaneous positivity
(most frequent 11 environmental and 9 drug allergens)
Atopic dermatitis (AD) n=100
Control: allergic contact dermatitis (ACD) n=100

	AD	ACD
Positivity with environmental allergens	67	117
Positivity with drug allergens	48	35
Total	115	152

MAJOR DIFFERENCES



Provocation test positivity
foodstuff: 19 (itch and/or exacerbation)

Antistreptolysin O titre increase 33

Blood eosinophilia 61

Effect of specific hyposensitization
with Bencard inhalative SDV vaccine
n = 50

I – Completely cleared
II – Better
III – Unchanged
IV – Worse

Intracutaneous positivity

Bacterial allergens	AD n=100	Control Microbial eczema n=100
Streptococcus Group C,G (Bencard)	17	39
Staphylococcus aureus (Bencard)	11	26
Gram-neg bacteria (Bencard F5)	14	34
Fungi		
Trichophyton (Bencard G1)	36	72
C albicans (Bencard)	30	56
Mixed moulds (Bencard A13)	14	24

Poster 1*a*.

Order of frequency of intracutaneous allergens:

Inhalant Group A1: house dust, cat fur, feathers, dog hair, horse hair

Inhalant Group A2: rabbit fur, cow hair, sheep wool, goat hair

Pollens: grass, tree

Mixed moulds: Aspergillus, Penicillium, Alternaria, Cladosporium

Order of frequency of food intolerance:

egg white, cereals, chocolate, fruits, cow's milk, cheese

Order of frequency of epicutaneous chemical allergens:

$K_2Cr_2O_7$, $CoCl_2$, IPPD, $NiSO_4$, PPD, formaldehyde, ol. turpentine, $HgCl_2$, MBT, colophony

Order of frequency of epicutaneous drug allergens:

Balsam of Peru, chloramphenicol, neomycin, vioform, parabens, wool alcohols, pix lithantracis, pix juniperi, Benzocaine

The number of cases of proved epicutaneous sensitization is smaller in atopic dermatitis than in a corresponding patient population with allergic contact dermatitis. The difference in allergen spectrum might be due to the fact in atopic dermatitis there is a decreasing trend in the frequency of exposure to environmental allergens due to active prevention, whereas exposure to drug allergens increases as a result of sustained local treatment.

Poster 1*b*.

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