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Itch and Atopic Dermatitis: Clinical and Experimental Studies

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CONTENTS

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ABSTRACT

The aims of the study were to develop and evaluate methods for quantitative measurement of itch, to investigate the perception of itch in patients with atopic dermatitis (AD), and to measure itch in such patients during treatment with H_1-receptor antagonists or cyclosporin A, thereby exploring possible mechanisms in the pathogenesis of itch in AD.

In a double-blind, randomized, placebo-controlled, cross-over study of 30 AD patients using a potent, topical, antipruritic corticosteroid, two methods for measuring itch both successfully detected the itch-relieving effect of the corticosteroid. The two methods comprised new portable data-loggers (Pain-Track) for continuous recording of itch, and conventional visual analogue scale (VAS) forms for retrospective recording. The main advantages of the Pain-Track method are possibilities for frequent sampling, surveillance of compliance, and analysis of a large amount of data.

Induction and measurement of experimental histamine-induced itch were studied in 38 healthy subjects. It was shown that pruritic stimuli should be presented in a random order so as to avoid systematic errors in the perception of itch. Two rating scales, a seven-stepped non-verbal scale on a Pain-Track logger, and a 100-mm VAS on a potentiometer, were found valid for continuous recording of itch.

The perception of experimental itch was studied in 32 AD patients and 32 healthy controls. The itch responses provoked by wool fibres were significantly stronger in AD patients than in controls, whereas the histamine-induced dose-response curves for itch did not differ significantly between the two groups, who discriminated equally well between weak and strong histamine stimuli. No increased skin mast cell releasability was shown in vivo to compound 48/80 in AD patients. Their itch responses to the different pruritic stimuli did not correlate with clinical itch intensity, eczema score or serum IgE-level.

In a double-blind, randomized, placebo-controlled, cross-over study of 25 AD patients, the effect on clinical itch of a sedative (clemastine) and of a non-sedative (terfenadine) antihistamine did not differ from that of placebo, although both drugs had a pronounced H_1-receptor-antagonizing effect in the skin and clemastine was significantly sedative. These findings support the view that histamine is not the major pruritogen in AD, and that sedation is not necessarily associated with itch relief.

In a double-blind, randomized, placebo-controlled, cross-over study of 10 AD patients, 10 days' treatment with cyclosporin A (CSA), 5 mg/kg/day, significantly reduced itch intensity, eczema score and the number of peripheral blood eosinophils. Relapses were seen within 2-30 days of completion of CSA therapy. In at least 50% of the patients, CSA reduced the number of CD3+, CD4+, HLA-DR+, IgE+, CD23+ (low-affinity Fc-IgE receptor+), intercellular adhesion molecule-1+, and EG2+ (activated eosinophils) cells in lesional skin. The changes of itch magnitude in the patients did not strictly parallel any specific change in the occurrence of these cell surface markers. The mechanism of action for the antipruritic effect of CSA remains unclear, but it is hypothesized that cytokines may be involved in the pathogenesis of itch in AD.

Key words: antihistamines, cyclosporin A, cytokines, eosinophils, histamine, lymphocytes, measurement, Pain-Track, pruritus, visual analogue scale.

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Wahlgren CF, Hägermark Ö, Bergström R, Hedin B. Evaluation of a new method of assessing pruritus and antipruritic drugs. Skin Pharmacol 1988;1:3-13.

II. Wahlgren CF, Ekblom A, Hägermark Ö. Some aspects of the experimental induction and measurement of itch. Acta Derm Venereol (Stockh) 1989;69:185-189.

III. Wahlgren CF, Hägermark Ö, Bergström R. Patients' perception of itch induced by histamine, compound 48/80 and wool fibres in atopic dermatitis. Acta Derm Venereol (Stockh) 1991; in press.

IV. Wahlgren CF, Hägermark Ö, Bergström R. The antipruritic effect of a sedative and a non-sedative antihistamine in atopic dermatitis. Br J Dermatol 1990;122: 545-551.

V. Wahlgren CF, Scheynius A, Hägermark Ö. Antipruritic effect of oral cyclosporin A in atopic dermatitis. Acta Derm Venereol (Stockh) 1990;70:323-329.

VI. Wahlgren CF, Tengvall Linder M, Scheynius A. Effects of cyclosporin A in atopic dermatitis: expression in the skin of IgE, low-affinity Fc-IgE receptors, ICAM-1 and eosinophilic granulocytes. Eur J Dermatol; in press.

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ABBREVIATIONS

AD	atopic dermatitis
cAMP	cyclic adenosine monophosphate
CD	cluster determinants
CSA	cyclosporin A
ECP	eosinophil cationic protein
EDN	eosinophil-derived neurotoxin
FPNVS	fixed-point, non-verbal scale
GM-CSF	granulocyte-macrophage colony-stimulating factor
ICAM-1	intercellular adhesion molecule-1
ID	itch duration
IL	itch latency
IL-	interleukin-
Imax	maximal itch intensity
MBP	major basic protein
PBS	phosphate-buffered saline
Tii	total itch index
TNF	tumour necrosis factor
VAS	visual analogue scale

INTRODUCTION

General aspects of itch

The definition of itch, or pruritus, as "an unpleasant sensation which provokes the desire to scratch" was introduced by Samuel Hafenreffer in 1660 (1) and is still used. Itch can be regarded as a 'physiological' and well-adapted sensation when it elicits a scratch response that removes potentially harmful stimuli from the skin, such as fibres, chemicals or parasites. When itch occurs as a symptom of disease it is considered 'pathological' and here there is no obvious advantage in scratching. On the contrary, scratching leads to further damage of the skin and may initiate a vicious 'itch-scratch' circle. Pruritus is the major symptom of many skin diseases, but it is also found in association with internal diseases, e.g. obstructive biliary disease, chronic renal failure, endocrine dysfunction and myeloproliferative disorders. Itch is often overlooked as a trivial problem, but it can be extremely distressing and as disabling as severe pain.

Most of our present knowledge concerning pruritus has been gained by investigations on human subjects. There is no established research model for itch in animals (2); even though they often scratch, their scratching behaviour is not necessarily specific for the itch sensation, as scratching may also be provoked by noxious stimuli probably associated with pain (3). Research in humans has focused mainly on experimentally-induced itch, where the conditions can be controlled, whereas less effort has been made to study clinical itch, presumably because of the difficulties of measuring such a subjective symptom.

The peripheral and central mechanisms of itch are incompletely understood. Itch can be elicited by chemical, mechanical, thermal and electrical stimuli (4, 5). No morphological structures have been identified as specific receptors for pruritus, but it is assumed that the 'itch receptor' is linked to free nerve endings (6). Shelley and Arthur used cowhage ('itching-powder'), i.e. spicules from the tropical plant mucuna pruriens containing a pruritogenic endopeptidase (mucunain), to show that the itch response could be elicited only in a limited zone close to the dermo-epidermal junction (4). Most C-fibres seem to end near the dermo-epidermal junction, but some penetrate into the epidermis (7). Pruritic stimuli induce activity in the thin, non-myelinated, slowly-conducting C-fibres (8, 9) that enter the ipsilateral dorsal horn of the spinal cord via the dorsal root. The activity set up in central neurons ascends contralaterally in the lateral spinothalamic tract, reaching the posterolateral ventral thalamic nucleus. From here, the activity is transmitted to the somatosensory cortex of the post-central gyrus (10). Itch can also be initiated centrally, e.g. by pathological processes in the central nervous system, such as surgical trauma (11), stroke (10, 12), brain abscess (13), brain tumour (14) and multiple sclerosis (15, 16).

The mechanisms of central processing and interpretation of itch are completely unknown, but undoubtedly the central nervous system can modulate the perception of itch. For instance, it is well known that emotions influence the itch sensation (17-20). The neuromodulation may act at different levels in the nervous system; one hypothesis is that descending fibres of the dorsolateral funiculus pathway terminating in the dorsal horn of the spinal cord can influence nociceptive transmission at spinal level (21). In 1965, Melzack and Wall proposed the 'gate-control' theory to explain why mechanical stimulation reduces pain sensation (22). They suggested that impulses

in afferent large-diameter myelinated A-fibres, activated by touch or vibratory stimulation, modulate and inhibit simultaneous impulses in C-fibres reaching the spinal cord, i.e. A-fibre input closes the 'gate' for C-fibre input of pain at spinal level. If this theory is applicable to itch, it may explain why mild mechanical stimulation of the skin transiently relieves mild pruritus. However, scratching is a fairly strong mechanical stimulus which activates nociceptors, and the nociceptive input appears to be particularly important for inducing the 'antipruritic state' caused by scratching (23). Furthermore, scratching may partially restore a central inhibition of itch (24).

The term 'itchy skin' (23) or 'alloknesis' (25) is used to describe a phenomenon of cutaneous hyperaesthesia in which itch is elicited by mild mechanical stimulation of the skin in an area surrounding a pruritic stimulus, such as a mosquito bite or the site of an experimental itching stimulus. In the experimental situation, the duration of the 'itchy skin', or 'alloknesis', exceeds the duration of the 'spontaneous' itching induced by e.g. an intradermal injection of histamine (23). The phenomenon of 'itchy skin' may be of considerable relevance for clinical itch in skin diseases, as it may explain why areas surrounding itching skin lesions are sometimes hyperexcitable by light mechanical stimulation, which is then perceived as itch. The neurophysiological mechanisms responsible for the phenomenon of 'itchy skin' are not known in detail, although the condition has several features in common with secondary hyperalgesia, which appears to be due to an altered central processing of mechanoreceptive input (26).

A number of peripheral inflammatory mediators have been investigated for their pruritogenic properties, but still very little is known as to what substances are responsible for the itching in various dermatoses and internal diseases. Peripheral pruritogenic mediators may act directly on the peripheral nerve endings, or indirectly by release and/or activation of endogenous substances that in turn stimulate the nerve endings. No principal or ultimate mediator of itch has been identified. Histamine is the most thoroughly studied pruritogenic mediator and was for long assumed to be the unique itch stimulus. Undoubtedly, histamine is the main itch-provoking substance in urticaria, although additional mediators may also contribute to the itch in this disease, but the role of histamine in other itching disorders remains to be defined. Other substances shown to be more or less pruritogenic in experimental studies are, for example, serotonin, proteases (e.g. trypsin, chymotrypsin, kallikrein and papain), peptides (e.g. bradykinin, sekretin, neuropeptides, such as substance P, vasoactive intestinal polypeptide, neurotensin and endorphins) and prostaglandins (PGE_1, PGE_2 and PGH_2). Most proteases and peptides are histamine liberators, and the itch they provoke can be blocked by antihistamines (27). However, kallikrein and papain induce itch without releasing histamine from dermal mast cells (28, 29). Prostaglandins seem to be modulators as they have no or weak pruritogenic properties on their own, but when they are given together with other pruritogens such as histamine or serotonin, the itch response is enhanced (30-32). Endorphins are endogenous peptides with morphine-like anti-nociceptive activity. They are weak pruritogens per se, but potentiate histamine-induced itch (33, 34).

Nothing is known about central mediators of itch, but it is speculated whether endogenous opioid peptides are involved as central transmitters of the itch sensation (34). Arguments supporting this

hypothesis are that itch occurs as a side-effect in patients receiving epidural or spinal opiates (35, 36), and that the opioid-antagonist naloxone has been reported to relieve such induced itch (35, 36), and also intractable pruritus of other origin (37).

Measurement of itch

There has been little work on the quantitative analysis of itch. This is in contrast to pain research, where many psychophysical investigations have been performed (38). Such studies have greatly contributed to the understanding of the pathophysiological mechanisms of pain and, not least, to our knowledge of the analgesic effects of drugs in various painful disorders. Consequently, the development and evaluation of quantitative models for analysing the itch sensation are of considerable importance.

In experimental studies, the itch-provoking stimulus can be controlled, which, of course, is impossible in the clinical situation. Therefore, very few attempts have been made to approach the intricate task of measuring clinical itch and the antipruritic effect of drugs. By definition, the itch sensation can only be assessed by the subject himself. This elementary fact disqualifies recently published studies in which itching was rated by someone else, e.g. the investigator or the parents of pruritic children. To circumvent the problem of quantifying itch intensity in experimental studies, most investigators have recorded the itch threshold (i.e. the lowest concentration of a solution provoking itch) or the itch duration. To rate the itch intensity, some kind of measurement scale must be applied.

Scales can be classified into four levels: nominal, ordinal, interval and ratio (39). The nominal scale comprises measurement at its lowest level, as it simply categorizes the property being scaled into different subclasses (e.g. red, blue, green). The next level is the ordinal scale, which gives a ranking of the scores without defining the differences between the scale steps (e.g. no, mild, moderate, severe). The interval scale determines the rank-order, but also has an equidistance between the scale steps. This allows the determination of equality of intervals or differences (e.g. temperature in °C or °F). The highest scale level is the ratio scale, which has all the characteristics of the interval scale, and, in addition, has a true zero point as its origin. This scale is the one most commonly encountered in physics (e.g. length, weight, time); it permits the determination of equality, rank-order, equality of intervals, and equality of ratios. The operations employed in obtaining the scores define and limit the manipulations and operations which are permissible in handling the scores. Thus, it is important to consider the level of the measurement scale.

Various types of rating scale have been used in the studies of clinical itch, but these scales have not been validated for this purpose. In recent years, however, some studies have focused on the evaluation of scales employed for assessment of experimentally-induced itch. Tuckett (40) applied a technique of ratio estimation of itch intensity in a study of healthy subjects, where itch was elicited by electrical stimulation of the skin. Pairs of electric stimuli were delivered: a control stimulus that remained unchanged throughout the experiment (10 Hz) and a test stimulus that was varied (2-100 Hz). The subject estimated the itch intensity of the test stimulus by comparing it with the itch intensity provoked by the control stimulus (e.g. 25% greater than, equal to, or 25% less than, the control). A significant dose-response relationship

was shown between the logarithm of the current frequency and the ratio of the test-to-control itch intensity. Simone et al. (41) used the magnitude estimation scale, i.e. an arbitrary numerical scale without an upper limit as described by Stevens (42), for intensity ratings of itch induced by intradermal injections of histamine in healthy subjects. Every 15th sec the subject assigned numbers of his or her own choosing, corresponding to the perceived magnitude of itch. To make the data easier to compare they were normalized in relation to a general mean value for all subjects and all histamine doses. It was concluded that the magnitude estimation scale gave a dose-dependent response for the itch intensity. Handwerker et al. used a 100-mm visual analogue scale (VAS) for rating itch intensity at 10-sec intervals during histamine iontophoresis (8). The stimulus was varied by changes in the current; the authors found that the perceived itch intensity correlated with the stimulus strength.

The use of the visual analogue scale (VAS) in the rating of subjective feelings is not a recent innovation, as it was introduced during the 1920's (43, 44). The VAS consists of a line with its boundaries defined as the extremes of the sensation to be rated. The subject rates by drawing a mark on the line, and then the marking is converted to a score by reading off the mark against a grid. Freyd listed the advantages of such a graphic rating scale; it is easily understood; it is quickly filled out and scored; and, does not require much subject motivation (44). Not until Aitken's work with a 100-mm VAS during the 1960's (45) did the use of this rating scale become more common in psychophysics. It has been extensively used and validated for the measurement of clinical and experimental pain (46), and also used but not validated for the assessment of clinical pruritus.

The qualitative aspects of the clinical itch sensation have not aroused much interest. For example, there is no 'itch questionnaire' analogous to the McGill pain questionnaire. This pain questionnaire contains, in addition to an intensity scale, qualitative verbal terms to be chosen as a specification of the pain experience (47).

Measurement of scratching

As direct, non-subjective measurements of itch cannot be made, some investigators have measured scratching, since this is the objective indirect correlate to itch. Most of the recording methods used have employed some kind of nocturnal limb-movement meter. Savin et al., who were the first to quantify scratching in human skin disease, studied scratching patients in a sleep laboratory by monitoring muscle potentials from forearms, electro-oculograms, electroencephalograms, submental electromyograms and closed-circuit television (48, 49). Felix and Shuster employed bed-leg vibration transducers and movement-sensitive meters (modified 'self-winding' watches) attached to the wrists and ankles of patients during sleep (50). In a study by Summerfield and Welch, the cumulative time of nocturnal limb movement was measured using electromagnetic movement-detectors secured to the limbs (51). Aoki et al. monitored nocturnal scratching with paper strain gauges attached to the hands of the patients, and then applied a computer system for analysing the data (52). Recently, Mustakallio and Räsänen described a movement-sensitive radar system ('scratch radar') to be positioned by the patient's bed at night (53, 54). This method has the advantage of requiring no physical connection to the patient.

General aspects of atopic dermatitis

Atopic dermatitis (AD) is a constitutional, pruritic, chronically relapsing inflammatory skin disease, frequently associated with an increased serum IgE-level. It predominantly affects children and young adults. The prevalence of AD in Sweden has been reported to be 8.3% for 7-year old children (55), and 2.3% and 3.8% for adolescent boys and girls, respectively (56). In general, the prevalence of AD in the industrialised countries has increased since World War II (57). AD rarely starts before the age of 2 months, but by the age of 5 years approximately 90% of the patients who will develop AD have manifested their disease (58).

As there is no laboratory marker for AD, the diagnosis is based on major and minor clinical criteria, where the major features are pruritus, typical morphology and distribution of the lesions, chronic relapsing course, and personal or family history of atopic disease (asthma, hay fever, AD) (59). Three age-dependent clinical phases of AD are recognized, although large variations occur. The infantile phase ($\leq$ 2 years) is characterized by acute to subacute eczema with erythema, papules, oozing and crusting distributed over the head, the trunk and the extensor surfaces of the extremities. During the childhood phase (3-11 years), the lesions are most often those of a chronic dermatitis with lichenification and scaling involving the flexures (elbows, knees, wrists, ankles, neck). With increasing age the face is less often involved, whereas the hands are not infrequently affected. The adolescent/young adult phase ($\geq$ 12 years) is essentially similar to that of later childhood with lichenification and scaling of the flexures and of the hands, and sometimes the face and the upper trunk are affected. However, in some patients a dry and scaling skin is the dominating feature.

The disease activity often fluctuates, with remissions and exacerbations, and several factors can aggravate AD, such as reduced humidity, sweating, mechanical and chemical irritants, bacterial and viral infections, allergens and emotional stress. There is a tendency towards spontaneous healing and most cases heal during childhood or adolescence; by the age of 30 few cases persist (58).

The aetiology of AD is not known, but it is presumed to be multifactorial. A genetic predisposition seems to be a prerequisite and the mode of inheritance is probably polygenic (58). The pathogenetic mechanisms are not understood. Investigations have shown several pharmacological and immunological abnormalities.

One pharmacological disturbance that has gained much attention is the elevated cAMP-phosphodiesterase activity in mononuclear leukocytes of patients with AD, leading to decreased cAMP levels (60, 61), which in turn may explain increased histamine releasability from basophilic granulocytes (61, 62).

As AD is associated with impaired cell-mediated immunity, many investigations have focused on the T lymphocytes. The number of T-suppressor cells in peripheral blood is lower in AD patients than in healthy subjects, leading to an increased T-helper/T-suppressor cell ratio of about 2:1 in AD patients (63-65). The skin infiltrates consist predominantly of activated T-helper lymphocytes (HLA-DR+, CD4+), and here, the T-helper/T-suppressor cell ratio is approximately 7:1 (65). Activated T cells can produce various lymphokines with different inflammatory and immunoregulatory properties, for example interleukin-4 (IL-4) and interferon-γ. IL-4 in vitro induces synthesis of IgE from B-lymphocytes and inhibits interferon-γ synthesis, whereas the IgE-inducing effect of IL-4 is antago-

nized by interferon-γ (66). AD patients show increased IL-4 mRNA expression in peripheral blood T cells (67), and an imbalance between IL-4 and interferon-γ may account for the increased IgE levels in AD patients.

The role of IgE in AD remains to be clarified. The frequency of elevated serum IgE-level varies: for example, in one study it ranged from 6% in patients with mild AD and no respiratory atopy, to 79% in subjects with severe AD and concomitant respiratory atopy (68). The IgE bound to mast cells and basophilic granulocytes is involved in the pathogenesis of the immediate hypersensitivity reaction, including the late-phase reaction, but it has not been proved that these reactions are involved in the pathogenesis of AD, although this has sometimes been discussed (69, 70). A possible role for IgE is suggested by the recent finding of IgE bound via low-affinity Fc-IgE receptors ($Fc_{\varepsilon}R2$/CD23) on Langerhans' cells in involved skin, and to a lesser extent in uninvolved skin, of AD patients with elevated serum IgE-levels (71, 72). Epidermal Langerhans' cells bearing allergen-specific IgE may bind and present allergens to T lymphocytes, inducing cytokine release and an inflammatory response (71, 73, 74). However, the T cell defect seems to be primary and the IgE abnormality secondary. The importance of the immune system and T cells in AD is supported by the fact that atopy has been transferred with bone marrow transplantation from an atopic donor to a non-atopic receiver (75), and conversely, that transplantation of normal bone marrow to patients with Wiskott-Aldrich syndrome has cured their eczematous lesions, which are indistinguishable from AD (76, 77).

Itch in atopic dermatitis

While the distribution and morphology of AD varies, the pruritus is a constant and dominating symptom, and AD patients judge the severity of their eczema more by the itch intensity than by the appearance of the skin lesions (78). The itch is usually intense but remittent with regular paroxysms in the evening and at night. It has long been debated whether the itch precedes the visible skin lesions in AD. At the beginning of this century Jacquet suggested that the itching was the primary cutaneous phenomenon, succeeded by the skin eruptions ("le prurit est prééruptif") (79). Others believe that the erythema is the first event (62), and this is supported by a study of AD patients by Graham and Wolf, who found that cutaneous flushing during experimental stress interviews was followed by itching and scratching (18). As the pathogenesis of itch in AD is still unknown, it is not understood why, for example, erythema, sweating, dry skin, or wool elicits itch in these patients. According to Rajka (58, 80) two components of itch can be distinguished in AD: first, itch provoked by various immunological or non-immunological stimuli, and secondly, an increased itch sensitivity in the skin with reduced itch thresholds or prolonged itch durations for pruritic stimuli.

The various immunological and non-immunological stimuli that elicit itch in AD are assumed to act directly on the 'itch receptor', or indirectly via subsequent release of pruritic mediators. The pruritic mediator or mediators of AD are not identified. Histamine has been a persistent candidate and is the most thoroughly studied pruritogen. Lewis reported in the 1920's that intradermal injections of histamine provoke redness, wheal and flare ('triple response'), accompanied by itching (81, 82). During the 1930's, Williams suggested that histamine might play a role in the pathogenesis of AD, as he found that intra-

muscular injections of histamine provoked increase of skin temperature in the flexures of AD patients, but not in the flexures of healthy controls (83). Reports of elevated histamine levels in both involved and uninvolved skin of AD patients were published during the 1960's (84, 85), but raised levels were also found in other forms of disseminated eczema (85). However, a more recent study of lesional AD skin showed no increase of cutaneous histamine levels (86). In a study of plasma histamine, increased levels were found in some, but not in all, AD patients (87).

Much interest has concentrated on basophils and mast cells, as these cells produce histamine and other inflammatory mediators. Peripheral blood basophils of AD patients are normal in number, but in vitro studies show abnormal function with increased (88, 89) or faster histamine releasability (90).

The microscopic features of AD skin vary with the nature of the clinical lesion. The acute lesion is characterized by epidermal intercellular oedema, and epidermal and perivascular dermal inflammatory infiltrates. The chronic lichenified lesion shows acanthosis and hyperkeratosis, and dermal inflammatory infiltrates in both perivascular and intervascular locations. The inflammatory cellular infiltrates are composed predominantly of lymphocytes and macrophages. The number of mast cells is greater in lichenified lesions, but not in acute lesions or in clinically normal skin (91-93). Only occasional neutrophils, eosinophils and basophils are noted (91, 92) Venular alterations are observed in both lesional and normal AD skin comprising endothelial cell hypertrophy and homogenous thickening of the basement membrane. Cutaneous nerves in lichenified plaques exhibit demyelination and fibrosis, but these morphological changes may be secondary to the repetitive trauma of scratching (91, 92).

The itch sensitivity in the skin of patients with AD has been investigated by experimental induction of itch. Using intradermal injections of histamine, Cormia determined the itch threshold in patients with various pruritic dermatoses. The threshold was lower in involved than in uninvolved skin, but only a single case of AD was included in the study (17). Arthur and Shelley found decreased itch thresholds for intradermally injected trypsin and a prolonged itch duration for cowhage spicules in lesional skin of the antecubital fossa of 5 patients with AD, compared with healthy controls (94). With intradermal injections of trypsin, Rajka showed that the duration of itch was prolonged (> 2 min) significantly more often in involved (95) and uninvolved skin (96) in 25 and 20 AD patients, respectively, than in controls with various other eczemas or psoriasis. However, in a study of the itch duration in lesional and non-lesional skin in 100 AD patients and 115 non-AD patients, Harnack concluded that the 'trypsin test' was not decisive in the differential diagnosis of AD (97). Heyer et al. recently used histamine iontophoresis at 6 different current intensities in non-lesional skin in 27 AD patients and 20 healthy controls, who rated the itch intensity on a VAS (98). It was shown that the slope of the dose-response curve for itch was significantly steeper in the controls than in the AD patients; thus, the controls perceived a significantly higher itch intensity to the strongest histamine stimulus than did the AD subjects. The latter were also, unlike the controls, unable to discriminate between weak and strong histamine stimuli. These authors' quantitative study of perception of itch intensity is in contrast to the previous findings of increased itch sensitivity in patients with AD.

Scratching in atopic dermatitis

Many of the skin lesions in patients with AD are secondary to scratching or rubbing of the skin. Lichenification and excoriations would not appear if the traumatization could be prevented. Itching evokes scratching that further damages the skin and increases the inflammation, which in turn leads to more itching, i.e. a vicious 'itch-scratch' circle is easily established and this is an important factor for the maintenance of symptoms in AD. Using a 'scratching machine', Goldblum and Piper showed that lichenification could be induced experimentally after 60 - 90 days of daily scratching during one hour per day, which was equivalent to a minimum of 140,000 scratches (99).

There is a large variety of scratching techniques, e.g. vigorous scratching with the fingernails, gentle rubbing with the palms, and scratching with mechanical tools, such as a hair brush, a comb or a towel. Several authors have pointed out various general psychological aspects of the scratching behaviour. It has been stated that scratching includes a component of pleasure (100), and that it may reflect somatization of emotions (101). Many patients tend to scratch during periods of emotional stress or frustration (18), but scratching during idleness is also very common, probably due to lack of distraction and social control. Moreover, scratching can be a conditioned response. In an experimental study by Jordan and Whitlock, it was shown that AD patients were more prone to develop a conditioned scratching response than were controls (102).

Studies of nocturnal scratching in sleep laboratories indicate the existence of a general scratching pattern valid not only for AD, but probably for all pruritic dermatoses (49). Scratching occurs during all stages of sleep, but the frequency with which the bouts of scratching start is related to the sleep stage. Scratching is more frequent in stages 1 and 2 than in stages 3 and 4 of orthodox sleep, whereas the frequency during paradoxical sleep (rapid eye movement) is in between (48, 49, 103). A reduction of deep sleep (stages 3 and 4) has been reported in patients with various disorders where scratching occurs, i.e. there is a relative increase of more superficial sleep, where scratching is more frequent (101). The duration of the scratching bout is fairly constant for each individual (approximately 10 s) and not related to the depth of sleep (49). Savin et al. found that 2 - 6% of AD patients' time asleep was associated with scratching (48), whereas Aoki et al. reported scratching during 15 - 38% of nocturnal recordings in AD patients (52). Scratching during sleep is performed predominantly with the hands, but is also associated with augmented restlessness noted as increased leg movements (50, 51). Itchy patients can make as much as 40% of their total 24-h movement during their 7.5 h of sleep, implying a considerable consumption of energy due to nocturnal scratching and restlessness (50).

General considerations

Itch is a dominating symptom in dermatology and the primary symptom in AD, but nevertheless, very few attempts have been made to develop and evaluate methods for its quantitative measurement. There are a few exceptions, as some recent studies have focused on methods for measuring itch intensity in experimentally-induced itch (8, 40, 41), but for clinical itch, practically no recording method has been evaluated or validated. This is a major shortcoming, as valid quantitative techniques for the assessment of itch would be of considerable value for studying the mechanisms of itch, and for the evaluation of antipruritic therapies.

The cause of itch in AD and its pruri-

togenic mediator(s) are still unknown. Most previous studies have revealed an increased itch sensitivity as measured by reduced itch thresholds or prolonged itch durations following pruritic stimuli, but in a recent study of itch intensity it was concluded that AD patients perceived less itch than did controls (98).

AIMS OF THE STUDY

The aims of the present study were:

(1)

to develop and evaluate methods for quantitative measurement of clinical and experimental itch,

(2)

to study the perception of itch in patients with AD, and

(3)

to measure itch in AD patients during treatment with H_1-receptor antagonists or the immunosuppressive drug cyclosporin A, thereby exploring possible mechanisms in the pathogenesis of itch in AD.

MATERIAL AND METHODS

Subjects

Patients with AD (I, III-VI)

Adult patients with persistent AD, fulfilling the criteria of Hanifin and Rajka (59), were recruited from those who had attended the Department of Dermatology at the Karolinska Hospital. A total number of 85 patients, 29 males and 56 females, participated in the investigations; 10/85 patients were involved in two of the studies, and 1/85 in three of them. Table I summarizes data for the participating AD patients. The majority, 65/85 (76%) had a past or present personal history of respiratory atopy; asthma in 3/85 (4%), rhinoconjunctivitis in 38/85 (45%) and both in 24/85 (28%). The median age at onset of eczema was less than 1 year (range 6 weeks to 37 years). The severity of eczema ranged from mild to severe on examination, but those who were included all suffered from chronic pruritus with a duration exceeding the previous year. Exclusion criteria were pregnancy or lactation, present history of urticaria, skin infection or any other disease than atopy, internal pharmacotherapy for the previous week, internal corticosteroids for the previous 3 months and UV therapy/sunbeds for the previous week (I) or month (III-VI). The studies were performed in the period October - April and were approved by the Ethics Committee at the Karolinska Hospital.

Table I. Patients with AD participating in the different studies. N = the number of patients; M/F = Males/Females; ARD = percentage of patients with past or present history of atopic respiratory disease; ND = Not done.

Study	N	M/F	Age (years) Median	Age (years) Range	ARD	Serum IgE (kU/l) Median	Serum IgE (kU/l) Range
I	30	6/24	22.5	19-57	77%	ND	ND
III	32	15/17	24	18-41	69%	330	2-8800
IV	25	9/16	24	17-42	80%	ND	ND
V-VI	10	7/3	24	22-42	90%	2550	60-8600

samples every 10th min. The 'itch profile' of a subject was calculated by averaging the subject's itch intensity on the hour during daytime, i.e. it was based on samples every 60th min. The daily compliance of the Pain-Track recording was calculated as the percentage of the patient's responses to the hourly buzzer. As an arbitrary choice, all days with a compliance of less than 70% were excluded. As no hourly signals were given during sleep, the compliance could not be assessed for the night recordings, but nights following days with compliance below 70% were also excluded.

Symtrack (V-VI)

Symtrack is a later version of the Pain-Track system. The main difference is that the knob with the seven-stepped scale (FPNVS) on the Pain-Track logger has been replaced by a lever sliding along a 100-mm VAS (0 mm = 'no itch', 100 mm = 'maximal itch') on the Symtrack logger (size 142 x 90 x 25 mm). The new logger has a recording capacity of 16 weeks at a sampling interval of 10 min (adjustable from 1 to 10 min).

VAS forms (I, III-VI)

The VAS forms were sheets of paper with a single horizontal 100-mm line with no other markings than the end points, which were defined as 'no itch' (= 0 mm) and 'maximal itch' (= 100 mm). The subjects were told that the mid-point of the scale should be half of the 'maximal itch'. The itch intensity was rated retrospectively over a period ranging from 12 h to 3 days by making a pen marking on the line. In study I, the patients rated the itch intensity of the previous day and night in the evenings and mornings, respectively. In studies III, V and VI, the clinical itch intensity was rated for the previous 24 h. In study IV, a 'global' rating of pruritus was made for each 3-day treatment period. The compliance of the VAS recordings was calculated as the proportion of forms completed.

Measurement of clinical sedation (IV)

The patients rated their 'global' clinical sedation for each treatment course in study IV by filling in forms with a 100-mm VAS. The end points were defined as 'no sedation' (= 0 mm) and 'maximal sedation' (= 100 mm).

Induction of experimental itch and flare (II-IV)

Itch was induced chemically by intradermal injections and mechanically by wearing a woollen sweater. Solutions of histamine hydrochloride (1.0, 3.3, 10 and 100 μg/ml) and of the histamine liberator compound 48/80 (10 μg/ml) were made by dilution with buffered saline, which also served as control in all experiments. All of these solutions were included in study III, whereas the three weakest, and only the strongest of the histamine solutions, were given in studies II and IV, respectively. Using double-blind technique and random injection order, 10 μl of each solution was injected intradermally into non-lesional skin of the upper arms. In addition, injections were given single-blind in non-random order in study II (see study protocol). The injection sites were gradually changed to avoid tachyphylaxis to histamine. After completion of the injections, a knitted woollen sweater with long sleeves was worn for 5 min in study III. The mean diameter (±SD) of the wool fibres was 33.4±7.9 μm (i.e. fibre thickness medium to coarse).

Measurement of experimental itch and flare (II-IV)

The intensity of the experimentally-in-

duced itch was monitored continuously for up to 15 min after the injections and for 5 min with the woollen sweater, using a linear potentiometer equipped with a 100-mm (II-IV) or 200-mm (II) VAS. The potentiometer lever, sliding along the VAS, controlled the position of a pen on a plotter out of sight of the subject. The end points were marked as described above. The time interval between injection and start/stop of itch was recorded as well as the perceived itch intensity. This allowed the calculation of itch latency (IL, sec), itch duration (ID, sec), peak value of itch (maximal itch intensity = Imax, mm), and a 'total itch index' (Tii = area under the curve on the plot, mm^2) reflecting both intensity and duration of itch. In cases where wool provoked itch (III), the subjects were asked to state whether or not the itching was accompanied by a pricking sensation. In study II, a Pain-Track logger with a seven-stepped FPNVS was used in some subjects instead of the VAS on the potentiometer. In this experimental situation, the Pain-Track sampling interval was changed from 10 min to 1 sec. The variables obtained were the same as with the potentiometer, although Imax and Tii were given in arbitrary units.

Five min after each injection, the flare response was outlined with a marking pen on the skin and traced onto transparent plastic film from which the area (mm^2) was calculated using a planimeter.

Blood chemistry (III, V-VI)

Serum IgE-levels were determined with radioimmunoassay. In the CSA study (V-VI), blood and urine samples were obtained before and during each treatment period. The blood samples were analysed for routine chemistry, number of peripheral blood eosinophils, and CSA whole-blood level (CYCLO-Trac™ (105)).

Skin biopsy specimens (V-VI)

Before, and on the last day of, each treatment period with CSA and placebo, 4-mm punch biopsies were taken from lesional and non-lesional skin, i.e. 8 biopsies per patient. The lesional biopsies were taken from symmetrical lichenified plaques (posterior axillary folds, arms and/or buttocks), 'before' on one side and 'after' on the contralateral. The non-lesional biopsies were obtained from the same areas. Biopsies from the same regions were taken at a minimum distance of 5 cm and during an intervening interval of 3-4 weeks. The biopsy specimens were divided into two parts, one for formalin-fixed, paraffin-embedded sections and one for frozen, acetone-fixed sections.

The formalin-fixed, paraffin-embedded, 3.5-µm-thick sections were used for routine histochemical staining and for immunoperoxidase staining. The specimens intended for frozen sections were kept in Histocon® (Histolab, Gothenburg, Sweden) at +4° (for less than 24 h) until they were snap-frozen in chilled isopentane and stored at -70°. The frozen, acetone-fixed, 6-µm-thick cryostat sections were used for immunoperoxidase staining and for double immunofluorescence staining.

Routine histochemical staining

Sections were stained with haematoxylin-eosin for estimation of cellular infiltrates, and with Luna's haematoxylin-Biebrich scarlet solution, which stained granules of the eosinophilic granulocytes. The number of eosinophilic granulocytes per Luna-stained section was counted.

Immunoperoxidase staining

A 3-step monoclonal antibody peroxidase-antiperoxidase (PAP) technique (106) was used. Endogenous peroxidase was blocked by incubation in 0.3% H_2O_2 in

phosphate-buffered saline (PBS) for 15 min and then the sections were allowed to react with normal rabbit serum (diluted 1/10) for 10 min to reduce non-specific staining. After this the sections were incubated with a panel of mouse monoclonal antibodies, as summarized in Table II. Rabbit anti-mouse immunoglobulin (diluted 1/40) was employed as a secondary antibody and finally the sections were allowed to react with preformed complexes of horseradish peroxidase and monoclonal anti-horseradish-peroxidase antibodies (diluted 1/250). The peroxidase reaction was developed with 3-amino-9-ethylcarbazole. The sections were counterstained with Mayer's haematoxylin. Specificity tests included omission of the primary antibodies and staining was not observed in these tests. The dilutions of the antibodies were determined using sections from normal lymph nodes and from normal and diseased human skin.

The sections were examined separately twice by two independent investigators under coded conditions. At least two sections per antibody and biopsy were examined. Sometimes the examiners' assessments showed minor differences, but as all the specimens were reexamined a third time and discussed by the two investigators together, a final common judgement was obtained before the code was released.

A cell was defined as positive (reactive) if it had a visible nucleus and staining all around the cell surface. Estimation of the total amount of positive cells per section was based on a semiquantitative scale (0 = no, + = few, ++ = moderate, +++ = many positive cells). The scale was adjusted so that the scale step 'many' (+++) referred to the maximal number of cells reactive to each antibody within all the specimens. After release of the code, the relative changes in the occurrence of reactive cells between 'before' (baseline) and 'after' (10th day of treatment) each period were expressed in terms of increase, decrease or no change. For EG2-stained sections, the number of eosinophils per section was counted. These quantitative data were used for comparison with the results of Luna's staining of eosinophils, and for analysis of the correlation with the number of peripheral blood eosinophils. In addition, the quantitative EG2 data were transformed into qualitative data by classifying each biopsy specimen into either of the two categories 'presence' or 'absence' of EG2-stained cells. These qualitative data were used for comparison of the patients' EG2 skin expression 'before' and 'after' each period.

Double immunofluorescence staining

Double immunofluorescence staining was performed on biopsy specimens from 4 patients selected on the basis of their having elevated serum IgE-levels (range 1900-6300 kU/l). Frozen, acetone-fixed, 6-μm-thick sections were incubated with mouse monoclonal anti-IgE-D1/D2 antibodies (see Table II; working dilution 1/10) for 30 min, followed by washes in PBS. The sections were then allowed to react with fluorescein isothiocyanate-labelled rabbit-anti-mouse immunoglobulin (working dilution 1/20) for another 30 min. Normal mouse serum was used as a blocking agent, and after this the sections were incubated with phycoerythrin-labelled anti-CD1a antibodies (T6/RD1, IgG1; staining Langerhans' cells; working dilution 1/20). The sections were mounted with glycerol in PBS containing paraphenylenediamine to reduce the fading of staining during microscopy (107). The double staining technique permitted the identification of IgE and CD1a in the same section, when the slides were analysed in a fluorescence microscope with interchangeable filter combinations

Table II. Specification of mouse monoclonal antibodies used for immunoperoxidase staining of skin biopsy specimens. MoAb = monoclonal antibody; CD = cluster determinants.

MoAb	CD	Reacting with	Working dilution	Source
anti-Leu-4	CD3	pan T cells	1:256	Becton Dickinson, USA
anti-Leu-3a	CD4	T-'helper/inducer' cells, some macrophages and Langerhans' cells	1:256	"
anti-Leu-2a	CD8	T-'cytotoxic/suppressor' cells	1:32	"
anti-IL-2r	CD25	IL-2 receptor ß-chain	1:16	"
anti-Leu-6	CD1a	Langerhans' cells	1:64	"
anti-HLA-DR		activated T cells, B cells, macrophages, monocytes, Langerhans' cells	1:128	"
anti-IgE-D1/D2		IgE	1:160	CG Magnusson, Karolinska Hospital
anti-$Fc_{\varepsilon}R2$	CD23	low-affinity Fc-IgE receptor	1:25	Dakopatts, Denmark
anti-ICAM-1	CD54	intercellular adhesion molecule-1 (ICAM-1)	1:80	Serotec, UK
EG2		activated eosinophils	1:5	Pharmacia, Sweden

for the fluorochromes. Staining was not found in control sections without the primary antibodies. The number of epidermal CD1a+ cells per section was counted, as was the number of cells per section that were both CD1a+ and IgE+. The numbers of positive cells were in the same range as those observed in control sections stained only for CD1a or IgE.

Statistical methods

Qualitative data, such as the quality of itch and the frequency of itch induction, were analysed with the χ^2 test (III).

For the experimental itch and flare responses, the Mann-Whitney U test was used for comparison between AD patients and controls (III), and the Wilcoxon signed-rank test for comparison between the periods in the antihistamine study (IV). For each of the experimental itch and flare variables, each subject's dose-response curve for histamine was computed using linear regression analysis. Student's t test was performed to compare intercepts and slopes between groups (III), and to analyse the significance of dose-response relationships (II-III). In addition, the histamine-induced itch and

flare responses of the AD patients were compared with those of the controls using analysis of variance (III). Multiple regression analysis was used to check for influence of age and sex on the experimental histamine reactions (III).

The clinical itch variables were analysed using parametric tests when an assumption of at least interval scaling and normal distribution were considered reasonable; otherwise non-parametric tests were performed. In study I, the distribution of the itch intensity scores was analysed using the Filliben probability plot correlation coefficient test (108). In spite of an assumption of normal distribution for the clinical itch variable, the scale level may be questioned in the measurement of sensations. Therefore, in general, both non-parametric and parametric tests were employed, but with the same results. The results presented for clinical itch were based on the following tests: sign test (I), Student's t test (I), analysis of variance (IV) and Wilcoxon signed-rank test (V-VI). The 'itch profile' was analysed with Student's t test (I), analysis of variance and orthogonal polynomial decomposition (IV).

In the antihistamine study (IV), sedation and consumption of hydrocortisone cream were analysed using analysis of variance. In the CSA study (V-VI), eczema score, hydrocortisone consumption, serum magnesium and number of blood eosinophils were compared using the Wilcoxon signed-rank test. Changes in skin expression of EG2+ cells 'before' and 'after' each period were analysed with a binomial test.

The correlation between the number of dermal EG2+ cells and the number of peripheral blood eosinophils was determined using Spearman's rank-order correlation coefficient (VI). In all other analyses of correlation, the product-moment correlation coefficient was used.

RESULTS

Data from introductory patient interviews (I, III-VI)

Several factors were reported to aggravate clinical pruritus in the 85 AD patients. Heat and perspiration provoked itching in 82/85 (96%) patients, wool fibres in 77/85 (91%), emotional stress in 69/85 (81%), certain foods in 42/85 (49%), alcohol in 37/85 (44%) and common colds in 31/85 (36%). The most pruritic period was reported to occur in the evening or at night in 62/85 (73%) patients, in the morning in 8/85 (9%) or at any time in 13/85 (15%). Itching had disturbed sleep frequently for the previous 3 months in 56/85 (66%) patients. Where bedrooms were shared, 37/56 (66%) patients reported that the sharers complained of the patient's nightly scratching. The use of mechanical scratching devices (e.g. towels, brushes, combs, back-scratchers, scissors) was reported in 32/85 (38%). The majority, 56/85 (66%) had tried antihistamines as antipruritic drugs, whereas a minority, 5/85 (6%) sometimes drank alcohol to relieve itching.

Dropouts (I-VI)

Twenty-six patients completed study I. Of the four dropouts, two were due to an exacerbation of the eczema during the placebo course, and two were due to technical disturbances within the Pain-Track data-logger. The other studies were completed by the participators, but in study III one AD patient refused to wear the woollen sweater, and one could not stand it for more than 85 sec (perceiving Imax = 96 mm). In study IV, one patient could not attend for her experimental histamine injections during one of the periods and in another case, a 7-day Pain-Track recording was lost because of a defective battery.

Measurement of clinical itch

Compliance (I, III-VI)

The compliance for the Pain-Track (I, IV) and Symtrack (V-VI) recordings is presented in Table III. In total, 831 days and nights were recorded by the AD patients using the computerized systems. Nineteen patients had one or more days with a compliance less than 70%, meaning that a total of 44/831 (5%) days and nights had to be excluded. A compliance of 100% was achieved on 345/831 (42%) days. The compliance tended to decrease towards the end of longer recordings, such as the 18-day recording by the patients in study IV.

All VAS form ratings performed at hospital visits were completed (III, IV). In studies I and V-VI, the patients themselves had to remember to fill in their VAS forms. In study I, these forms were completed for 401/416 (96%) days and nights, 3 and 9 patients not completing the VAS ratings for a total of 4 days and 11 nights, respectively. In studies V-VI, the daily VAS forms were completed for 220/240 (92%) days, 4 patients failing to fill in the forms for a total of 20 days. Thus, 35/656 (5%) VAS ratings that should have been made at home were not returned. There was a tendency to decreased compliance towards the end of periods for VAS ratings made in the mornings (I), while this was not seen for evening ratings (I, V-VI).

Ordinal-scaled variables (I)

Table IV shows that the Pain-Track median itch intensities during the two last days and nights were significantly lower with betamethasone dipropionate treatment than with placebo. The average percentage of time awake without pruritus on days 3-4 was 35.8% during the active therapy and 21.5% during the placebo course. This difference was statistically significant ($p<0.01$). For the VAS ratings, an antipruritic effect of the corticosteroid compared with the placebo was detected on days 3 and 4, and on night 4 (Table V).

Ratio-scaled variables (I)

As a hypothesis of normal distribution of the scores from the Pain-Track and the VAS form recordings could not be rejected, these data seemed to be sufficiently normally distributed.

The results from the Pain-Track recordings from days 3-4 and nights 3-4 are shown in Fig. 1. Twenty-four patients had a compliance level of at least 70% on both of these days. The average itch intensity was significantly lower with betamethasone dipropionate treatment than with placebo. The onset of the antipruritic action was rapid; a significant difference between the corticosteroid and the placebo was shown even on the first day of therapy ($p<0.05$), and for days 2-4 it was more pronounced ($p<0.001$). With

Table III. Patients' compliance in Pain-Track/Symtrack recordings of clinical itch.

Variable	Study I	Study IV	Study V-VI
Compliance (mean±SD)%	89.8±8.9	92.2±5.4	90.6±6.6
Compliance (range)%	66-100	82-99	80-99
Number of subjects with any day excluded	5/26 (19%)	9/25 (36%)	5/10 (50%)
Number of days excluded	17/208 (8%)	17/443 (4%)	10/180 (6%)
Number of days with 100% compliance	77/208 (37%)	198/443 (45%)	70/180 (39%)

Table IV. Results of sign tests based on ordinal-scaled variables from Pain-Track recordings in 24 patients on days/nights 3 and 4 of treatment with betamethasone dipropionate (BD) and placebo (PLO).

Median itch intensity	Number of patients day 3	day 4	night 3	night 4
BD > PLO	0	2	3	2
BD = PLO	12	9	5	5
BD < PLO	12	13	16	17
P value	<0.001	<0.01	<0.01	<0.001

Table V. Results of sign tests based on ordinal-scaled variables from VAS form recordings in patients on days/nights 3 and 4 of treatment with betamethasone dipropionate (BD) and placebo (PLO). N=number of subjects; NS=not significant.

Itch intensity	Number of patients day 3 (N=26)	day 4 (N=24)	night 3 (N=24)	night 4 (N=19)
BD > PLO	1	2	8	2
BD = PLO	0	1	2	1
BD < PLO	25	21	14	16
P value	<0.0001	<0.0001	NS	<0.01

the active therapy, the average itch intensity level decreased significantly from day 1 to 4 ($p<0.01$) in the 21 patients who had a compliance ≥70% for each of these days. The first night after beginning therapy there was no difference in itch intensities between the corticosteroid and the placebo, but there was a difference for the 2nd ($p<0.05$) and for the last two nights ($p<0.001$). There was also a difference between the first night of active therapy and the last ($p<0.05$). Fifty-four percent of the patients changed the intensity rate control during the night recordings, but nobody changed it every night. In general, changes were made during 18% of the night recordings. There was no correlation between the number of nightly changes and the itch intensity level.

The VAS scores from days 3-4 and nights 3-4 are shown in Fig.2. The average itch intensity was significantly lower with betamethasone dipropionate treatment than with placebo. A significant difference in itch intensity was shown between the corticosteroid and its indifferent vehicle on day 2 ($p<0.01$) and days 3-4 ($p<0.001$), but not on the first day of therapy. The average itch intensity level decreased significantly from day 1 to 4 ($p<0.01$) with active treatment in the 23 patients who completed their VAS forms for each of these days. The nightly levels of pruritus did not differ between the treatments until night 4 ($p<0.001$), and this was evidently due to increased itch intensity with placebo, as no significant difference was shown between the first night with the corticosteroid and the last.

Table VI. Correlation coefficients between scores from days/nights 3 and 4 within each method of itch recording during treatment with betamethasone dipropionate (BD) or placebo (PLO).

Method of itch recording	Days 3 vs 4		Nights 3 vs 4	
	BD	PLO	BD	PLO
Pain-Track	0.88	0.90	0.44	0.77
VAS forms	0.81	0.84	0.31	0.54

Correlations (I)

The correlation coefficients between the mean scores of Pain-Track and VAS forms were 0.71 ($p<0.001$) for days 3-4 and 0.72 ($p<0.001$) for nights 3-4 with betamethasone dipropionate, and 0.63 ($p<0.01$) for days/nights 3-4 with placebo. The correlations between day/night 3 and 4 within each method of assessment are shown in Table VI. The 'within' correlations were higher for the Pain-Track recordings than for the VAS form recordings. For both methods, the correlations within the night recordings were considerably lower than within the day recordings.

'Itch profile' (I)

The daytime 'itch profile' in 25 AD patients was obtained by averaging their itch intensities from samples on the hour during days 1-4. Days with compliance <70% were excluded. The itch intensity was significantly higher at 09.00 h and at 21.00 h than at 14.00 h for both betamethasone dipropionate ($p<0.05$) and placebo ($p<0.01$). As shown in Fig. 3, the morning and evening peaks were less pronounced during the active therapy.

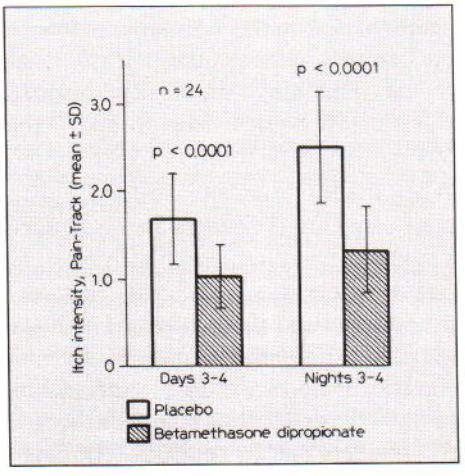

Fig. 1. The itch intensity measured with Pain-Track recordings during days 3-4 and nights 3-4 of treatment with betamethasone dipropionate or placebo in patients with atopic dermatitis.

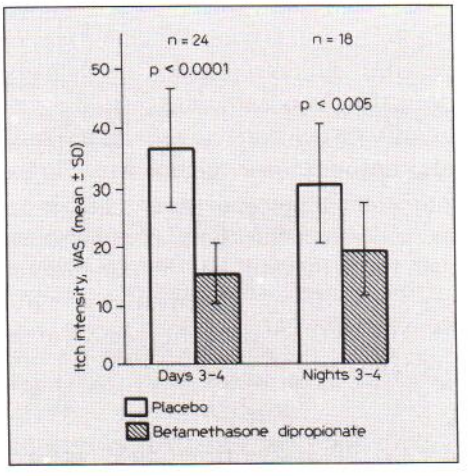

Fig. 2. The itch intensity measured with VAS form recordings during days 3-4 and nights 3-4 of treatment with betamethasone dipropionate or placebo in patients with atopic dermatitis.

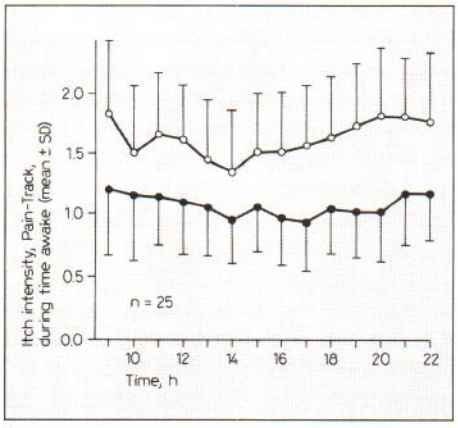

Fig. 3. The 'itch profile' measured with Pain-Track (samples every 60th min) during days 1-4 of treatment with betamethasone dipropionate (lower curve) and placebo (upper curve) in patients with atopic dermatitis.

Induction and measurement of experimental itch (II)

Increasing versus decreasing stimulus strength

When histamine was injected intradermally using stepwise increasing concentrations (1.0 - 3.3 - 10 μg/ml), significant dose-response relationships ($p < 0.001$) were shown for Imax and Tii, but not for IL or ID. When histamine was injected in the reverse order, i.e. stepwise decreasing concentrations (10 - 3.3 - 1.0 μg/ml), no itch variable showed a significant dose-response relationship. For example, the itch responses had higher numerical values for 1.0 μg/ml of histamine than for 3.3 μg/ml. For the flare response, a significant dose-response curve ($p<0.05$) was obtained irrespective of the injection order.

VAS versus FPNVS

For the VAS, significant dose-response relationships ($p<0.05$) were found for all of the itch variables (IL, ID, Imax and Tii), whereas for the FPNVS this was shown for ID and Tii ($p<0.05$). The flare responses showed significant dose-response curves ($p<0.01$) in both experiments.

Perception of experimental itch (III)

Experimental itch responses

The different injections did not induce an itch response significantly more often in AD patients than in controls, but the woollen sweater tended to do so (23/31 vs 16/32). The quality of the wool-provoked itch sensation, however, differed significantly ($p<0.01$) between the two groups, as 16/23 patients with AD but only 3/16 controls responding to wool felt 'pure' itch without a pricking sensation. In total, 197/256 (77%) histamine injections induced itching in the subjects and these histamine-induced itch responses were accompanied by visible flare reactions in 186/197 (94%) injections; whereas itching without visible flare responses occurred after 11/197 (6%) injections. The responses of the AD group and the control group did not differ in this respect.

The ID, Imax and Tii for histamine 10 μg/ml and for wool fibres were significantly higher in the AD patients than in the controls, whereas the itch responses for the remaining stimuli did not differ significantly between the groups. Table VII shows the Tii for the various stimuli. When the ID, Imax and Tii for all histamine concentrations were analysed using a repeated-measurement model, no significant differences were found between the patients and the controls. For histamine, linear regression analysis revealed significant dose-response relationships ($p<0.001$) for ID, Imax and Tii both in AD patients and in controls. The intercepts (α) and slopes (β) did not differ significantly between the two groups of subjects (Table VIII), showing that both patients and controls were able to discriminate between weak and strong hista-

Table VII. Total itch index (mean value±SD, mm^2) for AD patients (n=32, except for wool fibres, where n=30) and healthy controls (n=32). Recording time for wool fibres 5 min, for all other agents up to 15 min. P value denotes a significant difference between the patients and the healthy controls. NS=not significant.

Agent	AD patients	Healthy controls	P
Saline	206 ± 794	181 ± 598	NS
Histamine 1 µg/ml	791 ± 1083	730 ± 1705	NS
Histamine 3.3 µg/ml	2119 ± 2984	2569 ± 5033	NS
Histamine 10 µg/ml	7435 ± 10959	3065 ± 4636	<0.05
Histamine 100 µg/ml	12588 ± 17872	9206 ± 10003	NS
Compound 48/80 10 µg/ml	4689 ± 5089	4489 ± 6454	NS
Wool fibres	3630 ± 3902	1329 ± 3048	<0.01

mine stimuli, and that their discriminative ability did not differ significantly. The sex and age of the subjects did not significantly influence the experimental itch responses, although a tendency to reduced values was found with increasing age.

Experimental flare responses

The frequency of flare responses induced by the different injections did not differ significantly between the AD group and the control group. Flare reactions were observed after 228/256 (89%) histamine injections, and of these, 186/228 (82%) were accompanied by itching, while 42/228 (18%) induced a flare response without any itching. This pattern did not differ between the AD patients and the controls. Table IX shows that the two strongest histamine concentrations and compound 48/80 induced significantly smaller flares in the AD patients than in the controls. For histamine, a repeated-measurement model showed a significant difference between the two groups ($p< 0.05$). The histamine-induced flare reactions showed significant dose-response relationships ($p<0.001$) for both groups, but the slope (ß) of the flare-regression line was significantly ($p<0.05$) steeper in the healthy controls than in the AD patients (Table VIII). The flare responses induced by histamine and compound 48/80 were significantly larger ($p<0.01$) in females than in males in both the AD and the control groups. The age of the subjects did not influence the flare responses.

Table VIII. Mean values of the estimated intercepts (α) and slopes (ß) for the histamine-induced dose-response curves for itch and flare responses in 32 patients with AD and 32 healthy controls. Standard errors for the slopes in brackets. P value denotes a significant difference between the two groups. NS = not significant.

Variables	Intercepts (α)			Slopes (ß)		
	AD	Controls	P	AD	Controls	P
ID	60.8	39.5	NS	156 (23.9)	129 (21.2)	NS
Imax	14.3	8.1	NS	19.0 (2.6)	17.2 (2.2)	NS
Tii	254	204	NS	6229 (1699)	4193 (817)	NS
Flare	134	268	NS	518 (48.8)	691 (56.0)	<0.05

Table IX. Flare responses (mean ± SD, mm^2). P value denotes a significant difference between patients with AD (n = 32) and healthy controls (n = 32). NS = not significant.

Agent	AD	Controls	P
Saline	35 ± 108	20 ± 53	NS
Histamine 1 µg/ml	194 ± 333	295 ± 358	NS
Histamine 3.3 µg/ml	361 ± 475	634 ± 586	NS
Histamine 10 µg/ml	593 ± 627	892 ± 507	<0.05
Histamine 100 µg/ml	1211 ± 737	1679 ± 753	<0.01
Compound 48/80 10 µg/ml	556 ± 591	1040 ± 806	<0.01

Correlations

The AD patients' mean clinical itch intensity (±SD, mm) for the previous 24 h was 41.1±30.0 (range 1-91), the mean eczema score (±SD, arbitrary units) was 9.1 ±7.8 (range 1-30), and the mean serum IgE-level (±SD, kU/l) was 1563±2500 (range 2-8800). However, these variables did not correlate significantly with the Tii (injections, wool) or the flare responses (injections). Furthermore, neither in the patients nor in the controls did the Tii for the wool fibres and the Tii for any of the injections correlate significantly.

Effects of antihistamines (IV)

Experimental itch and flare responses

The results of the intradermal injections of histamine (100 µg/ml) are shown in Fig. 4. The histamine-induced itch and flare responses were significantly reduced by both terfenadine and clemastine compared with placebo (p<0.001). The experimental itch variables were all inhibited significantly more by terfenadine than by clemastine (p<0.05), whereas the inhibition of the flare reactions did not differ significantly between the two active drugs (p=0.07).

Clinical itch and sedation

The Pain-Track result based on samples every 10th min on days 2-3 of each period was used, as a pilot study showed that a steady-state for the inhibition of histamine-induced skin flare response had been reached during these days.

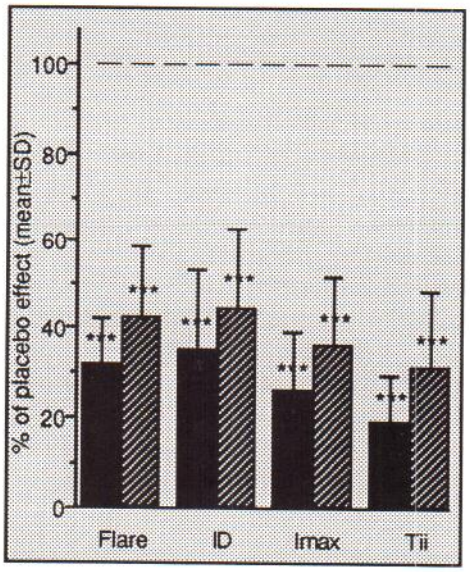

Fig. 4. The effect of terfenadine (60 mg b.i.d.) and clemastine (2 mg b.i.d.) on histamine-induced (100 µg/ml) flare and itch responses in patients with atopic dermatitis. The recordings were made on the third day of treatment, 5-7.5 h after the morning dose. The results of the histamine injections during the placebo period are defined as 100% (- - -). ■, Terfenadine (n=24); ▨, clemastine (n=25); ID=itch duration; Imax=maximal itch intensity; Tii=total itch index (see Material and Methods). ***= Significantly different from placebo (p<0.001).

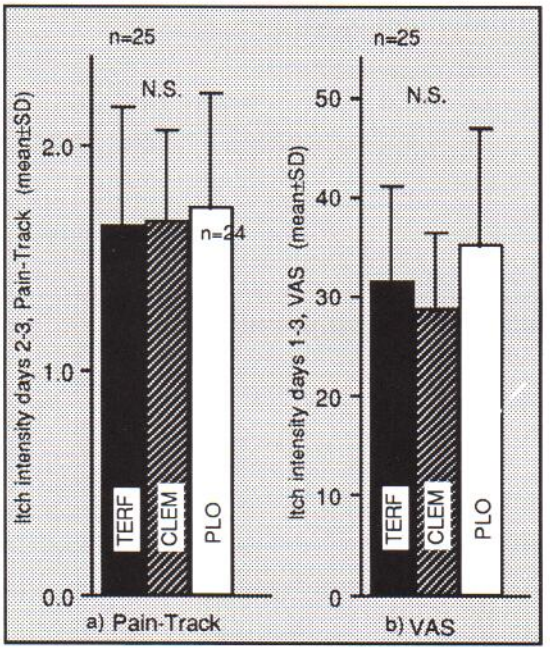

Fig. 5. The itch intensity measured with (a) Pain-Track (samples every 10th min) and (b) VAS forms, during treatment with terfenadine 60 mg, clemastine 2 mg or placebo b.i.d. in patients with atopic dermatitis. TERF=terfenadine; CLEM=clemastine; PLO=placebo; n=25, except for Pain-Track, recording during placebo, where n=24; NS= not significant.

These Pain-Track recordings showed that neither the mean clinical itch intensity (Fig. 5 (a)), nor the mean percentage of time awake without pruritus differed significantly between the three treatment courses. No decrease in itch magnitude appeared during the periods (baseline vs day 3) and the amount of topical hydrocortisone used during the washout after each period did not differ. In agreement with the Pain-Track recordings, the 'global' retrospective 3-day VAS ratings of clinical itch intensity did not differ significantly between the three treatments (Fig. 5 (b)). No order effect could be shown. The 'global' sedation (mean± SD, mm) was 8.9±9.5 for terfenadine, 38.9±17.3 for clemastine, and 13.9± 10.1 for placebo. Clemastine was significantly more sedative ($p<0.0001$) than terfenadine and placebo, whereas the two latter did not differ significantly from each other.

'Itch profile'

The 'itch profile' based on sampling on the hour did not differ significantly between the three treatments (Fig. 6). There was a significant increase in itch intensity during the day from 14.00 h ($p<0.001$). In most cases the maxima appeared within 60 min before sleep and were not inhibited by any of the antihistamines. Fig. 5 (a) and Fig. 6 are not contradictory, as the results are based on different sampling intervals (every 10th min during time awake, and every 60th min between 08.00 and 22.00 h, respectively).

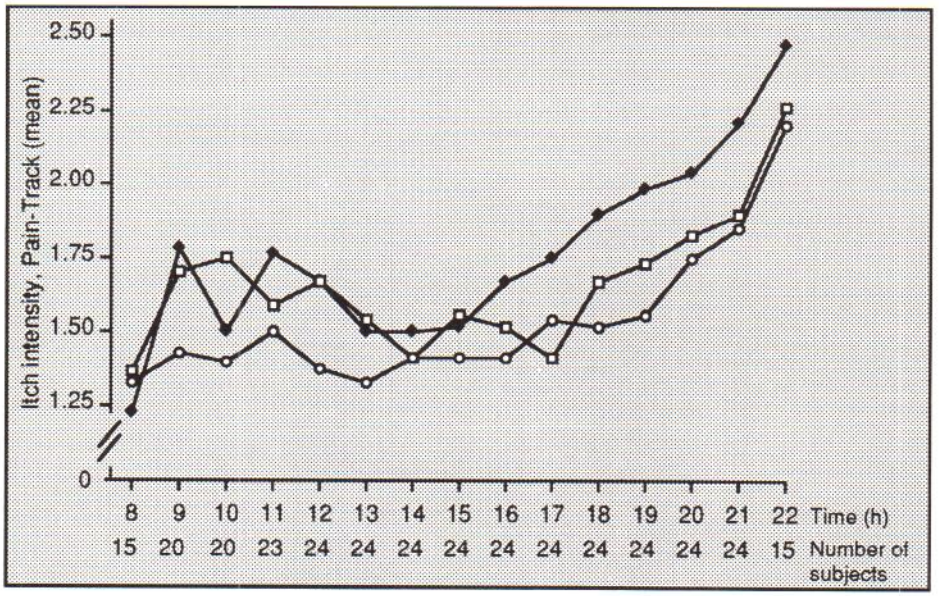

Fig. 6. The 'itch profile' measured with Pain-Track (samples every 60th min) on the last two days of treatment with terfenadine 60 mg (◆), clemastine 2 mg (□) or placebo (○) b.i.d. for three days in patients with atopic dermatitis.

Effects of cyclosporin A (V-VI)

Clinical itch and eczema

The results of the Symtrack and VAS form recordings were similar, as illustrated in Fig. 7. CSA significantly reduced the itch intensity on treatment days 9-10, when compared with the baseline value ('before') and also when compared with days 9-10 of the placebo period. The results of the Symtrack night recordings were similar. The patients' average 'itch profile' between 09.00 and 22.00 h on days 9-10 was on a lower level with CSA than with placebo. An analysis of the percentage of time awake without pruritus on days 9-10 of CSA treatment showed that 4 AD patients were completely free from itch, 2 spent more than 90% of the day without itch, while 4 were never free from itch. On days 9-10 of the placebo period, 9/10 patients were never free from itch. The onset of the antipruritic effect of CSA varied. Six patients had an effect within days 2-4, while in 3 others the decrease in pruritus was recorded in days 8-10. However, in one patient there was no itch relief during the 10 days of active treatment.

CSA treatment improved all patients, including the one where no itch relief was achieved. The eczema score was reduced significantly on the 10th day of active therapy, both when compared with the baseline value ('before') and when compared with the placebo value on day 10 (Fig. 8). No significant changes in eczema score were observed after the placebo period. The consumption of topical hydrocortisone 1% was significantly less ($p<0.01$) with CSA (48.5±47.3 g) than with placebo (90.7±70.0 g).

A gradual relapse of itch and eczema was observed in all patients 2-30 days after the completion of active treatment, but no severe rebound phenomenon occurred. On follow-up visits after 2 and 8 months, when most patients used semipotent/potent topical corticosteroids and UV therapy, the average eczema score

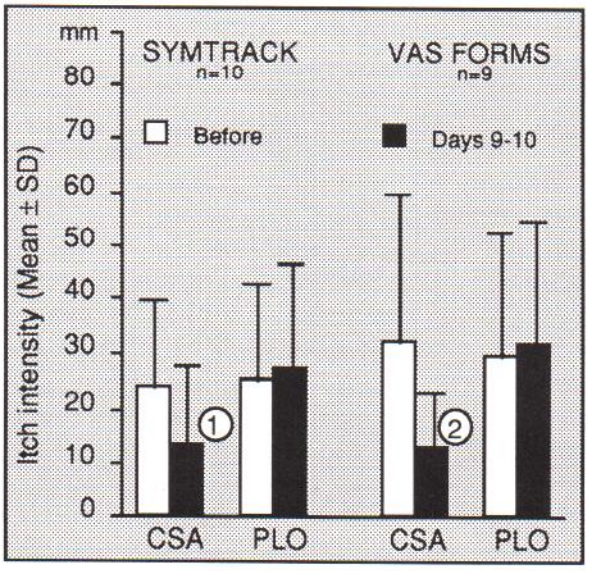

Fig. 7. The itch intensity recorded with Symtrack and daily VAS forms 'before' and during days 9-10 of treatment with cyclosporin A (CSA) 5 mg/kg/day or placebo (PLO) in patients with atopic dermatitis. (1) = significant decrease in relation to CSA 'before' ($p=0.01$) and to PLO days 9-10 ($p<0.05$). (2) = significant decrease in relation to CSA 'before' ($p=0.02$) and to PLO days 9-10 ($p<0.01$).

was 14.6±6.3 and 16.6±9.8, respectively. Some of the patients had a peripheral blood eosinophilia on these occasions, but otherwise no laboratory abnormalities were found.

Side-effects and laboratory findings

No severe side-effects occurred during the short-term, low-dosage CSA treatment. After some days with CSA, two patients reported mild hand tremor and another mild transient burning sensations in the hands and feet, the latter being also reported by a patient while on placebo. CSA induced a non-symptomatic, temporary but significant ($p<0.01$), decrease in serum magnesium from the baseline value (mean±SD) of 0.79±0.065 mmol/l, down to 0.65 ± 0.032 mmol/l on day 10. The number of peripheral blood eosinophils was reduced significantly ($p<0.01$) with CSA (baseline value: 514.6 x 10^6± 211.6 x 10^6/l; day 10: 279.5 x 10^6± 185.1 x 10^6/l). No significant changes were seen in serum creatinine or urea. All subjects had taken CSA and no toxic CSA blood levels were found. The average CSA whole-blood concentration 9-21 h after drug intake was 106.5±43.6 ng/ml.

Histochemical and immunohistochemical findings

Before the two treatment periods, lesional skin biopsy specimens showed small-to-large cell infiltrates, located mainly perivascularly in the dermis, as judged from haematoxylin-eosin-stained sections, except for one patient who displayed almost no cell infiltrates in 3 of 4 specimens. The majority of the infiltrating cells were CD3+, CD4+ and HLA-DR+, whereas CD8+ or CD25+ cells were less frequent. The numbers of IgE+ cells or CD23+ cells ranged from few to many and no lesional specimen was deficient in these cells. In general, the IgE+ cells were dendritic in the epidermis and non-dendritic in the dermis. The majority of epidermal and dermal CD23+ cells were non-dendritic, although several dendritic CD23+ cells were seen in the epidermis. The estimated proportion of IgE+ or CD23+ cells in the infiltrates ranged from 10-50% and 10-65%, respectively. The keratinocytes were HLA-DR negative,

DISCUSSION

Measurement of clinical itch (I)

Itch is a sensation that can be assessed by the patient himself, but not by any objective method. To circumvent this problem, several investigators have recorded nocturnal scratching, which can be measured objectively; but such recordings have been restricted to inpatients due to the technical facilities that are required. Scratch recordings have sometimes been validated by correlating them to subjective assessments of itch (50, 51). This highlights the problem: ultimately one has to rely upon the patients' ratings of their subjective symptom. Therefore, it is essential to develop and evaluate valid itch-recording methods that can be used by outpatients in everyday life.

Betamethasone dipropionate, a potent topical corticosteroid with a well-known antipruritic effect in AD patients (109), was used as a tool to evaluate two different methods of itch recording: continuous Pain-Track recording and retrospective VAS form recording twice daily.

The patients' compliance for the Pain-Track and VAS form recordings was about equal. In general, the patients regarded the VAS forms as easier to handle, but the majority believed that the Pain-Track recordings were more accurate. A major difference between the two methods is that the compliance can be monitored continuously with Pain-Track, increasing the authenticity of the itch recording. Moreover, the Pain-Track method avoids retrospection and the previous recordings are concealed from the patient; and a large amount of observations can be stored and analysed. The VAS ratings are retrospective and may be highly influenced by the itching during the minutes before filling in the form, and furthermore, there is a possibility for the patient to complete the ratings from memory just before returning the forms to the investigator. In the present study, the VAS ratings were performed in parallel with the microcomputer-based itch recordings, which may have increased the patients' inclination to follow the VAS form instructions properly.

The Pain-Track loggers were equipped with a seven-stepped FPNVS, whereas the rating forms had a 100-mm VAS. The level of the scale of measurement is a critical point for the choice of the statistical method to be applied in the analysis of data. For obvious reasons it is impossible to state definitely what level the two rating scales belong to. Strictly speaking the FPNVS and the VAS are both on an ordinal level, i.e. they give rank-orders between the scale steps, but the differences between the steps are not definable. On the other hand, with the non-verbal nature of the scales and with the operational instructions given to the patients (see Material and Methods), it is fair to say that the scales are on an approximate ratio level with equidistance between the scale steps and a true zero point. Ordinal-scaled data must be analysed with non-parametric methods, whereas ratio-scaled data may be analysed with parametric methods provided that the additional assumptions (e.g. the normal distribution of data) underlying the particular models or tests are valid.

In the present study, the patient acted as his/her own control and the variable to be analysed was a difference between some aggregate measure of the observations. If the scale is considered to be ordinal, it is not meaningful to compute means or differences. The methods available are now drastically reduced to processing a variable that uses only ordinal properties. e.g. the median, and the remaining test is a simple sign test. As an

alternative to using the median, the percentage of time awake without pruritus was determined from the Pain-Track recordings of each subject and period (days 3-4). Assuming approximate ratio scaling of the FPNVS and VAS, the average itch intensities were computed. As these were considered to be normally distributed, the parametric Student t test was applied. In experimental studies of pain measurement in humans, the VAS can be regarded as an approximate ratio scale (46), and parametrical methods are often used for the analysis of data obtained with fixed-point scales or VAS in pain measurements (110).

Using both non-parametric and parametric statistical methods, it was shown that both the Pain-Track recording with its seven-stepped FPNVS and the 100-mm VAS form recording were sensitive enough to detect the antipruritic effect of the topical corticosteroid in this double-blind, randomized, placebo-controlled study. No absolute, final judgement of validity can ever be given for the measurement of a subjective sensation (45), as the 'true' intensity of the sensation cannot be determined objectively. The validation has to be established through empirical observations. The fact that both itch-recording techniques detected the antipruritic effect of active therapy, and the finding of high correlations between the scores of the two techniques, support their validity for measuring clinical itch intensity. The high correlations between days 3 and 4 within each method support reliability. The lower correlations between the nights may reflect a more variable pruritus during that time or less accuracy in the night recordings. The itch intensities of the night recordings made with Pain-Track often reflected the itch intensity level just before falling asleep; 12/26 (46%) patients never changed the intensity rate control at night, and in cases where such changes were made these were not frequent (approximately every 5th night). Therefore, it may not be fully relevant to include night recordings with Pain-Track in the evaluation of clinical itch and antipruritic effect of drugs.

Induction and measurement of experimental itch (II)

The occurrence of systematic errors in the perception of experimentally-induced itch was studied by investigating the influence of the order in which various histamine concentrations were presented to the subjects. Our findings strongly support a random injection order. When histamine was injected in gradually decreasing concentrations no significant dose-response curves were obtained for the itch variables. The explanation might be that an initially higher concentration of histamine triggers the activation of modulatory events in the central nervous system and that this disturbs the perception of itch for subsequent weaker stimuli.

Scales for rating of sensations are sometimes criticized as they transform the sensation into an artificial unidimensional experience. However, this has to be accepted for practical reasons, and furthermore, in our study the only dimension that we focused on was the itch intensity. Several rating scales including the VAS have been validated for measurement of pain (38, 46, 111-114), while very few attempts have been made to validate scales for measurement of itch (8, 40, 41). For this reason, two different rating scales, the seven-stepped FPNVS and the 100-mm VAS, were investigated for their capacity to produce significant dose-response curves for itch responses induced by histamine injections. The VAS gave significant dose-response relationships for all of the four

experimental itch variables, whereas the FPNVS gave significant dose-response curves for two of them, the ID and Tii. The Tii is of greatest importance as it reflects both the intensity and the duration of itch. For both the FPNVS and the VAS, the Imax to the strongest histamine concentration (10μg/ml) was approximately 30% of 'the worst itch ever imaginable'. In contrast to the VAS, the FPNVS failed to give a significant dose-response curve for Imax, indicating that the seven-stepped scale was not sensitive enough to discriminate between the peak values of itch induced by the different low doses of histamine. The VAS has theoretically an infinite number of scale steps, but is usually divided into mm-steps. This does not necessarily imply that it is more sensitive than a scale with fewer scale steps (114). In sensory perception (e.g. auditory loudness, pain intensity), opinions differ as to how many steps of stimulus strength an individual is able to discriminate (112, 114-116) and this is of importance for the design of valid rating scales. Our present findings show that both the FPNVS and the 100-mm VAS can be used for continuous rating of itch intensity. As the VAS discriminated better for some of the itch variables it was considered of interest to provide the next version of the Pain-Track logger (Symtrack) with this scale.

Perception of experimental itch (III)

Various stimuli were used for inducing experimental itch. Histamine was chosen as it is the most thoroughly studied pruritogen. The histamine liberator compound 48/80 was included to investigate skin mast cell releasability in vivo. Wool fibres were employed as they provoke itch by mechanical stimulation and wool-provoked itch is of clinical importance to many AD patients (59, 117, 118).

The itch response induced by wool fibres differed significantly in quality and quantity between the patients and the controls. The AD patients more often perceived a 'pure' itch without a pricking sensation and the itch response was stronger than in the controls. This is in agreement with previous reports of an increased itch sensitivity in patients with AD (94-96). However, the itch responses provoked by the different histamine concentrations did not show such a clear-cut difference between the two groups of subjects, as only one of four concentrations (10 μg/ml) induced a significantly increased itch response in the AD patients compared with the controls. The remaining three concentrations gave itch responses that were only slightly and non-significantly increased in the patients. For example, for the Tii evoked by histamine 100 μg/ml (Table VII), about 150 subjects per group would have been needed for the observed difference to be significant at the 5% level, assuming a standard deviation of 15,000 in each group. Furthermore, the ability to discriminate between the different histamine concentrations did not differ between the patients and the controls. These data are in conflict with those of Heyer et al. (98), who used histamine iontophoresis to induce pruritus and found that the itch response to the strongest histamine dose was lower in AD patients than in controls, and that AD patients, unlike controls, were unable to discriminate between weak and strong histamine stimuli. Because the skin barrier is defective even in normal-looking skin of AD patients (119), it may be difficult to achieve a well-defined histamine dose close to the dermo-epidermal junction zone, where the itch response is assumed to be maximal (4) and this might explain the discrepancy between our results and the

findings of Heyer et al.

The wool-provoked itch was less than the itch provoked by the two strongest histamine concentrations, which was unexpected for the AD patients. Thus, a pruritic stimulus at a single point was perceived as stronger than pruritic stimuli over a larger area, which, in addition, was often eczematous. This may reflect the different modes of pruritogenic stimulation that were used, but on the other hand, nothing is known about how AD patients or healthy subjects process and perceive itch sensations provoked by many simultaneous pruritic stimuli. It is worth noting that the Tii induced by wool fibres did not correlate significantly with the eczema score. For example, the patient who perceived the highest Imax to wool (96 mm), was in fact the patient with the lowest eczema score. The possibility of conditioned itch responses to wool must be considered, but against this are the findings that the Tii elicited by the injections did not correlate significantly with the severity of eczema, and that four of the patients did not perceive itch, although they had reported in the introductory interviews that wool usually provoked itch. The latter may, of course, be due to the diameter of the wool fibres used in our experiment; the thicker the fibre diameter, the more pronounced the itch response (118). As the Tii and the eczema score did not correlate significantly, the question arises as to what happens to the itch sensitivity when the AD is clinically healed. Rajka found a prolonged ID to intradermal injections of trypsin in patients with healed AD and a current 'atopic rhinitis' (96), whereas patients with only 'atopic rhinitis' did not differ from controls (58). It is therefore possible that the increased itch sensitivity in AD patients persists for a long time, maybe for life. If this is true, it may reflect a primary constitutional abnormality in the perception of itch, or a secondary phenomenon of increased itch sensitivity due to the previous experience of an itchy skin disease, or both.

In vitro studies of basophilic granulocytes from patients with AD have shown an increased (88, 89) or faster (90) histamine release. Because mast cells are generally assumed to be functionally analogous to basophils, the skin mast cells may also have an abnormal histamine release, although this remains to be proved. In our present study, no significantly increased non-immunological (non-IgE-dependent) skin mast cell releasability was shown in vivo, as compound 48/80-induced itch and flare reactions were not more pronounced in AD patients than in controls. This finding is consistent with a previous study by Ruzicka and Glück (86), who found no difference between AD patients and controls in histamine release from skin sections challenged in vitro to compound 48/80.

The flare responses were smaller in AD patients than in controls. This is a well-known phenomenon (120, 121), but its cause is unknown, as is the reason for the larger flare reactions in females than in males. The difference in flare size between females and males emphasizes the importance of sex-matching when comparing flare responses between various groups of subjects. Skin reactivity to histamine is reduced by repeated intradermal injections of small doses of histamine (81, 122) and increased histamine levels in non-lesional atopic skin (85) may theoretically induce tachyphylaxis to histamine and lead to smaller flares. However, this seems less probable, at least for the free nerve endings of C-fibres, as the histamine-induced itch responses were not decreased in our AD patients compared with the controls. Intradermal injections of the neuropeptides substance P and neurotensin induce

equal itch responses, but induce smaller flares in patients with AD than in non-atopic controls (123), i.e. the neuropeptides produce a response similar to that of histamine in the present study. Several studies indicate an increased tendency to vasoconstriction in the skin of AD patients (120, 124) and this phenomenon may explain the reduced flare responses in these patients. After the 'healing' of the AD, the flare is normalized (121).

Effects of antihistamines (IV)

Our study of AD patients showed no effect on clinical itch of terfenadine or clemastine compared with placebo, although clemastine was significantly sedative. With intradermal injections of histamine, a peripheral H_1-receptor antagonizing effect was shown for both the active drugs, as they inhibited the itch and flare responses significantly compared with placebo. Thus, the patients had taken the drugs as requested and significant concentrations were achieved in the skin. If histamine was a main pruritogen in AD, as in urticaria, the antihistamines would relieve clinical itch promptly. It can be argued that our itch-recording methods were not sensitive enough to detect an antipruritic effect. However, as described above, both the Pain-Track method with its FPNVS and the 100-mm VAS forms were sensitive enough to detect an antipruritic effect of a potent corticosteroid in a placebo-controlled study (I), and further, both itch-recording techniques discriminated between the itch responses induced by various low doses of intradermally injected histamine (II). We are not aware of any method for recording clinical itch that has been validated as extensively as this one has.

There is still doubt as to whether histamine is involved in the pathogenesis of pruritus in AD. The assumption that histamine is the main pruritogen in this disease is based on several findings in AD patients concerning histamine levels in plasma and skin, number of skin mast cells and and abnormal histamine release, as reviewed in the present introduction. However, there are several arguments against histamine being the main itch-provoking substance in AD. First, when histamine is injected into the skin, itching is nearly always (see Results) accompanied by a visible 'triple response', i.e. local redness, whealing and an axon-reflex-mediated flare reaction (81, 82). As very low doses of intradermally injected histamine are urticariogenic but not pruritogenic (125), it is not probable that histamine provokes itching without concomitant visible changes in the skin. The pruritus in AD is not associated with whealing. Secondly, repeated intradermal histamine injections lead to the development of tachyphylaxis in the skin, i.e. further histamine injections do not induce any vascular response or itching (81, 122). In urticaria, where histamine is the major pruritogen, the itching of the individual wheals is transient, lasting for some hours, while new itchy lesions develop at new sites. Such tachyphylaxis is not compatible with the chronic pruritus of the individual lesions in AD. Thirdly, the antipruritic effect of antihistamines in AD patients is not satisfactory, whereas their antipruritic effect in urticaria is generally prompt.

Based on the results from an open study of the effects on pruritus and nocturnal scratching of sedative and non-sedative antihistamines, and of a sedative benzodiazepine, in 23 patients with various itchy dermatoses, Krause and Shuster (126) suggested that the antipruritic effect of antihistamines in non-whealing disorders is due to a sedative-related effect, although inhibition of itch is not a

general property of all sedatives (126, 127). Savin et al. (128) investigated the effects of trimeprazine, a sedative phenothiazine-related antihistamine, and of trimipramine, a sedative and antidepressive drug, on nocturnal scratching and sleep in patients with AD. They found that the time spent in stage 1 of sleep, when scratching is most frequent, was reduced by both drugs, and this explained the drug-induced, modest reduction of nocturnal scratching. In our study, clemastine was significantly sedative, but nevertheless, its effect on clinical pruritus did not differ from that of terfenadine or placebo. Sedation at night can relieve anxiety and insomnia, and the patients may worry less about their itching, but this is not necessarily associated with relief of itch.

The study of clinical pruritus and the antipruritic effect of drugs is a very difficult task for the clinical investigator. Several studies have been published concerning the antipruritic effect of antihistamines in AD, but most can be criticized as they do not fulfill the essential design criteria for the clinical evaluation of antipruritic drugs. Such criteria have been listed (129-131) and they, of course, include the use of a double-blind, randomized study design. Furthermore, placebo must be included as itching is a subjective symptom which can be highly influenced by any medication, active or not (132, 133). In our study, we preferred a cross-over design with the patient as his own control to minimize the importance of individual factors, such as severity of eczema, flare factors influencing the course of AD, and the subjective use of the itch-recording scales. Finally, it is important to define the basis for patient selection and to describe carefully the itch-recording method that is employed.

When studies not satisfying the essential demands of a controlled and well-conducted investigation are excluded, few remain to review. In a double-blind, randomized, placebo-controlled cross-over study, Savin et al. (134) found that the new non-sedative H_1-receptor antagonist LN2974 failed to reduce the itching recorded with VAS forms or the nocturnal scratching recorded with limb-movement meters in ten adults with AD. Using daily VAS ratings, Frosch et al. (135) studied the antipruritic effect of chlorpheniramine alone, chlorpheniramine plus cimetidine, and placebo, given for four weeks each in a double-blind, randomized, cross-over trial with 16 AD patients. The active treatments did not relieve itch better than placebo. Doherty et al. (136) employed a double-blind, randomized, placebo-controlled, but parallel, study of the antipruritic effect of 10 days' treatment with one of the two non-sedative antihistamines terfenadine (180 mg/day) and acrivastine (24 mg/day), or placebo, in 49 AD patients, 44 of whom completed the study. Itching, rated with VAS forms on entry and after seven days, was significantly reduced by each of the active drugs compared with placebo and it was concluded that histamine has an active role in the pruritus in AD. In contrast to these results, but supporting our present findings, Berth-Jones and Graham-Brown (137) recently reported the failure of 7 days' treatment with terfenadine (240 mg/day) to relieve itch in a double-blind, randomized, placebo-controlled, cross-over study of 24 AD patients, who rated their itch intensities on VAS forms twice daily on the last four days of each treatment.

We conclude that our present study (IV), in common with most previous carefully controlled studies (134, 135, 137), supports the view that histamine is not of major importance in the pathogenesis of itch in AD patients. Antihistamines may, in addition, to their H_1-re-

ceptor antagonistic action have other important effects. For example, terfenadine in vitro inhibits the release of histamine from basophils (138) and mast cells (139), and of leukotriene C_4 production from eosinophils (138), ketotifen in vitro inhibits histamine release from mast cells (139), loratadine in vitro inhibits release of histamine and leukotriene C_4 from mast cells (140), and cetirizine in vitro inhibits chemotaxis of eosinophils (141) The possible relevance of these experimental findings to the relief of clinical pruritus in patients with AD is still completely unknown and remains to be determined.

Effects of cyclosporin A (V-VI)

In recent years cyclosporin A (CSA), a cyclic polypeptide with potent immunosuppressive effects, has been used for the treatment of various inflammatory dermatoses (142-144) and several case reports have been published on the improvement of eczema and itching in patients with AD treated with oral CSA for 1 -31 months in doses of 1-6 mg/kg/day (145-152). CSA has also been reported to suppress pruritus promptly in patients with therapy-resistent Sézary syndrome (153) or mycosis fungoides (154). Therefore, we decided to investigate further the effect of CSA in a placebo-controlled, short-term study of AD patients. Within 10 days of CSA therapy an antipruritic effect was achieved in 9/10 patients and the eczema scores decreased in all 10, i.e. also in the patient who did not experience any itch relief. An interesting clinical observation was that CSA markedly reduced the erythema in the AD patients. The association between erythema and itching in patients with AD has often been pointed out (18, 62). Sowden et al. (155) recently confirmed the efficacy of CSA given for 8 weeks to AD patients in a placebo-controlled study. Because of the risk of serious side-effects the clinical use of CSA must be restricted to cases with refractory and severe AD not responding to other therapies, such as photochemotherapy, systemic corticosteroids or azathioprine.

The mechanism of action of CSA in AD is not yet understood. A major effect of CSA is the inhibition of IL-2 production from T-'helper' cells (142, 156-158) and this inhibition occurs at the level of IL-2 mRNA transcription (157, 159-161). By inhibiting IL-2 production, CSA suppresses the activation and subsequent proliferation of 'helper' and 'cytotoxic' T cells. In addition, CSA inhibits the production of other T-cell derived cytokines, such as interferon-γ, IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (142, 158, 159, 162). Furthermore, CSA affects a variety of other cells; for example, CSA in vitro has an antiproliferative effect on keratinocytes (163, 164) and endothelial cells (163), and inhibits the release of histamine from human lung mast cells (165). Thus, it cannot be stated that the effects of CSA in patients with AD depend merely on its immunosuppressive action on T cells.

The serum IgE-level is not decreased by CSA therapy (148, 151, 155). Munro et al. (151) in an open study of CSA-treated AD patients with an allergy to house-dust-mite antigen (HDM) showed that the immediate and late-phase reactions (6 h) to intradermally injected HDM were significantly increased, whereas the responses at 24 and 48 h did not differ from pre- or post-CSA treatment intradermal tests. In another open study, Higgins et al. (166) showed that the skin response to intradermally injected histamine was significantly increased in patients treated with CSA. Thus, CSA in

vivo does not seem to inhibit the release of, or the response to, skin mast cell-derived mediators. Since CSA produced a marked clinical improvement of AD, whereas the prick tests were not inhibited (151), the relevance of the immediate/late-phase type-I hypersensitivity reactions in the pathogenesis of AD seems doubtful.

Our immunohistochemical findings in pretreatment skin biopsy specimens fitted well with previous reports (63-65). We found that CSA reduced the skin expression of several of the cell surface markers. The mechanism of action for these events cannot be stated, but as CSA inhibits the production of various cytokines, some theoretical speculations are possible. A CSA-induced inhibition of the IL-2 production from T-'helper' cells may explain the reduction in the numbers of CD3+, CD4+ and CD25+ cells in the AD lesions. An inhibition of the interferon-γ production from T cells may account for the CSA-induced reduction in the number of HLA-DR+ cells. The expression of ICAM-1 on keratinocytes, occurring in several inflammatory dermatoses with dermal accumulation of T lymphocytes (167, 168), is stimulated by interferon-γ and tumour necrosis factor (TNF)-α and -β (167); a CSA-induced inhibition of these cytokines may explain the observed decrease in the number of ICAM-1+ keratinocytes after CSA therapy. In the majority of our patients, CSA decreased the expression of cell-bound IgE and low-affinity Fc-IgE receptors (CD23) in lesional skin. As in previous reports (71, 72), the serum IgE-level seemed to influence the number of cells bearing IgE. We did not follow the serum IgE in our patients, but as CSA did not reduce the serum IgE-levels in AD patients in recently published studies (148, 151, 155), we suggest that CSA down-regulates the CD23 expression in lesional AD skin, leading to reduced low-affinity binding of IgE. Hypothetically, an inhibition of IL-4 and/or interferon-γ may explain this phenomenon, as the expression of CD23 on e.g. human Langerhans' cells is induced by these cytokines (169).

Patients with AD often reveal a peripheral blood eosinophilia. The eosinophils contain, in their granules, toxic proteins: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO). On stimulation, the eosinophils degranulate and release these proteins into the circulation and the tissue. Increased serum levels of MBP (170) and ECP (171), and deposition of MBP in lesional skin (172) have been observed in AD patients. Cutaneous eosinophils also occur in patch tests with specific aero-allergens in patients with AD (173). We found that CSA reduced the number of peripheral blood eosinophils and also the number of patients with EG2+ cells present in lesional skin biopsies. In vitro studies show that human T lymphocytes from patients with blood eosinophilia on IL-2 stimulation can produce IL-5 and that IL-5 stimulates eosinophil colony formation (174). Experiments in the rat confirm that CSA can abolish hypereosinophilia (175). The role of the eosinophilic granulocyte in AD is, however, unknown. MBP in vitro induces histamine release from human basophils and rat mast cells (176) and when MBP, ECP or EDN are injected intradermally into human skin a wheal and flare reaction is provoked (177). However, it is unlikely that this mechanism is of importance for the induction of clinical itch in AD patients.

From the present study we cannot conclude that the observed findings are specific to CSA therapy. On the contrary, it is possible that they occur in any

type of effective treatment of AD. For example, some parallels can be drawn to treatment with corticosteroids; oral prednisone improves AD, but has no effects on serum IgE-levels (178), and furthermore, topical corticosteroids decrease the ratio of IgE+ to CD1a+ epidermal cells (72). In our present study, the changes of itch intensity in the patients did not strictly parallel any specific change in the immunohistochemistry, i.e. the patients who had complete itch relief on days 9-10 of the CSA treatment did not reveal any distinct uniform pattern of immunohistochemical changes when compared with the others, including the one where no itch relief was achieved with CSA.

In many pruritic skin diseases, such as AD and lichen planus, a mononuclear cellular infiltrate consisting predominantly of T lymphocytes is observed in the papillary dermis near the junction area, where the itch receptors are assumed to be situated (4). On activation, these lymphocytes produce various cytokines, i.e. soluble mediators with a large number of inflammatory and immunoregulatory effects. Cytokines are also produced by various other cell types in the skin, e.g. keratinocytes, macrophages, fibroblasts, endothelial cells and mast cells. Cytokines interact in a network, and the final response is determined by a cascade of complex interactions. The cytokines involved in AD remain to be determined and it is not known whether this disease has a specific pattern of cytokine release and interaction. Patients with AD have elevated serum levels of the IL-2 receptor (179-181). This presumably reflects the degree of lymphocyte activation and is found in many diseases with increased immunoreactivity. In vitro studies show decreased production of interferon-γ (182), TNF-α (182-184), IL-1 and IL-2 (184) from stimulated peripheral blood mononuclear cells of patients with AD. The proportion of peripheral T cells able to produce IL-4 is higher in atopic patients than in non-atopic controls (185). IL-4 in vitro induces synthesis of IgE from B-lymphocytes and inhibits interferon-γ synthesis, whereas the IgE-inducing effect of IL-4 is antagonized by interferon-γ (66, 186). An imbalance in the IL-4/interferon-γ ratio may explain the increased IgE production in AD patients. Antigen-specific cloned murine T cells can be divided into two groups based on the cytokine profile they produce; T_{H1} produce IL-2 and interferon-γ, and T_{H2} produce IL-4 (187). It is not proved that such dichotomized subsets exist in healthy humans, but in vitro studies show that less than 5% of human CD4+ T cells express IL-4 mRNA, whereas 60% of CD4+ T cells express IL-2 and interferon-γ messages (188). Techniques for culturing skin-infiltrating lymphocytes obtained via skin biopsies from lesional AD skin have recently been introduced and offer an exciting possibility of studying the functional activity and spectrum of cytokine production of lesional skin lymphocytes (189-191). In vitro-expanded cultures of AD-skin-derived lymphocytes, stimulated with mitogens or allergens, produce IL-4, GM-CSF and TNF-α, whereas the production of interferon-γ is reduced or absent (190, 191).

Whether cytokines can induce pruritus has not been studied systematically, but, interestingly, pruritus occurs as a side-effect of some cytokines that have been administered to patients. Injections of human recombinant GM-CSF in some cases induced local erythema and pruritus at the injection sites (192), or a pruritic widespread cutaneous eruption (192, 193). When intradermal injections of human recombinant IL-2 (30-90 kU) were given to patients with lepromatous lepro-

sy to reconstitute cell-mediated immunity in their cutaneous lesions, an induration of the skin was provoked at the injection site (194) and this reaction was accompanied by itching (Kiessling, personal communication). Further, Gaspari et al. (195) reported that cancer patients receiving high doses of intravenous IL-2 (30-100 kU/kg/8 h) frequently developed erythema and pruritus of the skin after 2-3 days' therapy. Immunohistochemical investigation of the skin revealed the presence of activated T lymphocytes in papillary dermis, and blood samples showed that the patients had developed a peripheral blood eosinophilia (195). These findings are similar to some of those encountered in AD patients. As the IL-2-provoked erythema and itching were not reduced by indomethacin or antihistamines, it was concluded that prostaglandins or histamine were unlikely to be the major cause of these symptoms (195). However, to complicate further this subject it was recently reported that infusions of IL-2 (10-20 kU/kg/8 h) improved eczema and pruritus in an open study of 6 children with AD (196), so the effects of human IL-2 injections may vary, possibly due to different routes of administration, doses and basic diseases.

The pruritic mediator or mediators, and their cellular origin, are still unknown in AD, as is the mechanism of the antipruritic action of CSA. However, since cytokine therapy can provoke itch as a side-effect and since CSA inhibits the production of various cytokines, it is hypothesized that such mediators may be of importance, either directly or indirectly, in the pathogenesis of pruritus in AD. To confirm or reject this hypothesis, experimental investigation of the pruritogenic properties of different cytokines is necessary, alone and in combination, as these substances interact.

PRINCIPAL FINDINGS

By using a drug with known antipruritic effect in patients with AD, we have shown that continuous recording with a computerized logger (Pain-Track) with a seven-stepped FPNVS, and retrospective recording with a 100-mm VAS on sheets of paper, can be used for quantitative measurement of clinical itch and for the detection of antipruritic effects of drugs. The main advantages of the Pain-Track method are that it avoids retrospection; that previous recordings are concealed from the patient; that frequent sampling is possible; that compliance can be surveyed, increasing the authenticity; and that a large amount of data can be stored and analysed.

In the induction of experimental itch, pruritic stimuli should be presented in a random order so as to avoid systematic errors in the perception of itch. Both the seven-stepped FPNVS and the 100-mm VAS were found valid for the continuous measurement of experimentally-induced itch.

The itch responses provoked by wool fibres, but in general not those provoked by histamine injections, were significantly stronger in AD patients than in healthy controls. This supports a hypothesis of increased itch sensitivity in AD patients, although this may not be valid for all pruritic stimuli. Histamine induced significant dose-response relationships for the itch variables both in patients and in controls. The ability to discriminate between weak and strong histamine stimuli did not differ between the two groups. No increased skin mast cell releasability was shown in vivo, as compound 48/80-induced itch and flare responses were not increased in AD patients compared with controls. The experimental itch and flare responses in the AD patients did not correlate significantly with clinical itch

intensity, eczema score or serum IgE-level.

Both clemastine and terfenadine had a pronounced H_1-receptor-antagonizing effect in the skin of AD patients, but their effect on the patients' clinical itch did not differ from that of placebo, although clemastine was significantly sedative. These findings support the view that histamine is not of great importance in the pathogenesis of itch in AD and that sedation is not necessarily associated with itch relief.

Cyclosporin A (CSA) in a dose of 5 mg/kg/day significantly reduced the itch intensity, the eczema score and the number of peripheral blood eosinophils in AD patients. No serious side-effects occurred, but relapses of clinical symptoms were seen within 2-30 days of completion of CSA treatment. In at least 50% of the AD patients, CSA reduced the number of CD3+, CD4+, HLA-DR+, IgE+, CD23+, ICAM-1+ and EG2+ cells in lesional skin. We suggest that CSA down-regulates the expression of low-affinity Fc-IgE receptors (CD23), which in turn may explain the decrease of the IgE expression on cells, including Langerhans' cells. The changes of itch magnitude in the patients did not strictly parallel any specific change in the immunohistochemistry. The mechanism of action for the antipruritic effect of CSA remains unclear, but as it is known that cytokine therapy can provoke itch as a side-effect and that CSA inhibits the production of cytokines, it is hypothesized that cytokines may be of importance, directly or indirectly, in the pathogenesis of itch in patients with AD.

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