

Time-course studies of immediate and delayed immune reactivity in patients with atopic dermatitis treated with herbal drugs

U. SØDERBERG¹, B. WINDELBORG NIELSEN², C. SCHADE LARSEN³, P. O. SCHIØTZ² & K. THESTRUP-PEDERSEN¹

Departments of ¹Dermatology, ²Pediatrics and ³Infectious Medicine, University of Aarhus, Denmark

We followed a number of *in vitro* parameters in nine adult patients with atopic dermatitis for a period of 3 weeks during which the patients took herbal drugs that are claimed to improve chronic eczema. The following investigations were performed weekly: blood leucocytes, circulating eosinophils, serum IgE, mitogen responsiveness of lymphocytes, interleukin 1 release, soluble interleukin 2 receptor levels in serum, and *in vitro* histamine release from basophils in blood. The study demonstrated increased levels of IgE in six patients, increased levels of soluble interleukin 2 receptor in six patients, and a significant correlation between the amount of IgE in serum and the maximal release of histamine from basophils in blood. In this open study five patients experienced a clinical worsening of their disease during the intake of herbal medicine. All other parameters were within normal levels. There was no significant change in the *in vitro* parameters during the observation period.

Key words: atopic dermatitis; basophils; histamine release; herbal extracts; IgE; interleukin 1; interleukin 2 receptors; mitogen responsiveness.

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It is well known that patients with atopic dermatitis (AD) can have remarkable variability in their disease activity. We have followed a number of *in vitro* parameters in a group of nine adult patients who took herbal drugs for their skin disease during the observation period. We wanted to see whether such drugs could influence the clinical disease activity or change immune parameters since they are frequently recommended and used by patients with AD (3). We are not aware of similar studies in atopic eczema.

PATIENTS AND METHODS

Patients

Nine adult atopic patients participated in the study following informed consent; five women

aged 18-40 years and four men aged 22-29 years. All had life-long AD and most of them had previously been in-patients due to exacerbation of their disease. The study took place from February to March 1989, when all were out-patients. Their skin disease was considered stable at the start of the study.

The study was an open, phase I study and approved by the Ethics Committee. It was conducted during a 3-week period with a total of four clinical and laboratory investigations. The patients used topical steroid (hydrocortisone butyrate; Locoid®) and emollient. During the first week they took Silica tablets, each containing 175 mg of dried water-extract from 875 g *eq-visetum arvense* (seaweed), in a dosage of 2 tablets t.i.d., and the following week 5 ml t.i.d. of a 70% alcoholic extract of AOH 176 tea

leaves (brand name). During the third week only topical treatment was given.

The clinical assessment included a semiquantitative score by the doctor, evaluating dryness, redness, infiltration, and excoriations of the skin. Photographs were scored blindly following the study, and the patients scored their subjective symptoms every week on a linear scale. The use of topical steroid was measured by weighing the tubes. The patients were also asked to monitor their morning peak flow using a hand-held vitalograph.

Laboratory investigations

We performed weekly investigations of the following parameters starting before herbal drugs were given: leucocytes, circulating eosinophils, serum IgE, histamine release from blood leucocytes, mitogen responsiveness, interleukin-1 release *in vitro*, and soluble interleukin-2 receptor in serum.

In vitro histamine release was measured using a glass microfibre-based technique as described in detail elsewhere (17). Briefly, samples containing 10 ml of whole blood were washed four times in PIPES-buffered saline solution to remove serum and anticoagulant (Na-EDTA 10 μ M). Microfibre-coated 96-well microtiter plates (Lundbeck Diagnostics A/S, Copenhagen, Denmark) were pretreated with 50 μ l of PIPES-buffered saline, and aliquots of 50 μ l washed blood were added to each well in 2 plates. To the wells of plate 1 were added a standard panel of secretagogues in 20 μ l of PIPES-buffered saline, namely formyl-methionyl-leucyl-phenylalanine (Sigma, St. Louis, MO, USA) (fMLP; 10^{-8} , 10^{-5} , and 10^{-3} M), Concanavalin A (Pharmacia, Uppsala, Sweden) (ConA; 12.5, 25, and 50 μ g/ml) and Rabbit-anti-human IgE (Behringwerke, Marburg, West Germany) in final concentrations of 0.033, 0.33, 3.3, 16.7, 33.3, 167 and 333 U/ml.

To the wells of each row of plate 2 was added 10 μ l of PIPES-buffered saline containing a preincubation agent, aminophylline (2 mM), nor-dihydro-guanoetic-acid (NDGA; 10 μ M),

acetylsalicylic acid (ASA; 0.55, 1.4 and 5.5 mM), and phorbol 12-myristate 13-acetate (TPA; 10 and 50 ng/ml). After a preincubation period of 10 min, at 37°C, anti-IgE at concentrations of 0, 0.33, 33, and 333 U/ml was added.

For quantitation of histamine, standards of 0, 5.2, 10.4, and 20.8 ng/ml were added to unstimulated aliquots of blood on both plates, generating linear curves by analysis of regression. Plates were incubated for 60 min at 37°C, and histamine release was measured fluorometrically as described (17).

Mitogen reactivity. Mononuclear cells were isolated from heparinized venous blood using Isoopaque-Ficoll, washed and dispensed in RPMI 1640 with 10% human, heat-inactivated serum in flatbottomed microtiter plates in a total concentration of 2×10^5 cells per well. PHA (Wellcome HA15) was added in concentrations of 0.1 and 0.3 μ g/ml, and Con A 0.125 μ g/ml, which were optimal for lymphocyte transformation. DNA synthesis was measured during the last 4 h of a 48 h cultivation period with ^{3} H-thymidine (19).

Interleukin-1 assay. Mononuclear cells (1×10^6 /ml) were isolated as above and incubated for 24 h with or without LPS 10^{-5} M in LPS-free RPMI 1640 (Gibco 12F2161) with 1% human, heat-inactivated serum and 25 mM HEPES. Supernatants were collected and assayed for IL-1 activity using the C₃H mouse thymocyte assay as described elsewhere (8).

Interleukin-2 receptor levels. Sera were collected from patients and controls (healthy laboratory personnel) and stored at -20°C until assayed. The concentration of soluble interleukin-2 receptors (sIL2R) was determined by an enzyme-linked immunosorbent assay (ELISA) as supplied in kit form (Cellfree® Interleukin-2 Receptor Test kit, T Cell Sciences Inc., Cambridge, MA) and used according to the manufacturer's instructions (13, 14). sIL2 concentrations are expressed in units/ml (U/ml) and 1,000 units is defined as the amount of sIL2R present in 1.0 ml of a reference prep-

aration of culture supernatant from PHA-stimulated peripheral blood cell lines. The detection limit is approx. 50 U/ml.

Statistics

Wilcoxon test for two samples was used. Kruskal-Wallis analysis of variance was employed to compare results from the 4 weekly determinations. Correlation coefficients were calculated by Spearman's Rank method. A $P < 0.05$ was considered significant.

RESULTS

Five of nine patients experienced increased disease activity during the observation period, whereas the remaining four were unchanged (Table 1). The physician and photo score paralleled this observation and the consumption of topical steroid increased on average from 20 g to 40 g per week in these five patients. The peak flow, leucocytes, and circulating eosinophils were not changed.

The serum IgE was increased in six patients; five of them experienced a flare of disease activity (Table 1). The increased IgE was unchanged during the observation period except in two patients, who had a drop in their IgE from 2,717 to 891 kU and 2,667 to 933 kU, respectively.

The remaining three patients had normal IgE levels throughout the study and no clinical flare.

The *in vitro* histamine release showed a large inter-individual variation. One patient did not release detectable amounts of histamine with any stimulant studied. Week-to-week variations in histamine release were relatively small in most patients (Fig. 1) and did not coincide with ingestion of the herbal drugs.

The maximum histamine release upon challenge with anti-IgE correlated significantly with serum IgE (Spearman's $r = 0.66$, $P < 0.001$; Fig. 1). The sensitivity to anti-IgE (Fig. 2) was not correlated to total serum IgE, the maximal histamine release occurred within a narrow anti-IgE dose range in all patients. The maximal ConA-induced histamine release was almost identical to the maximal anti-IgE-mediated histamine release (Spearman's $r = 0.93$, $P < 0.001$), whereas fMLP could only induce a measurable histamine release in four patients. Preincubation with aminophylline reduced the anti-IgE mediated histamine release to the level of spontaneous histamine release in all patients (Fig. 3). The lipoxygenase inhibitor NDGA, at 10 μ M, did not alter anti-IgE histamine release significantly, whereas the cyclooxygenase inhibitor ASA increased histamine release dose-dependently in the presence of anti-IgE. At an ASA concentration of 5.5 mM, a slight, but

Table 1
Interleukin-1 from LPS-stimulated mononuclear cells (units/ml)

Patient	Week no.				IgE value	Clinical worsening
	1	2	3	4		
LA	73	66	135	22	196	+
LHF	66	66	140	32	221	+
KBP	31	67	56	83	783	-
ILS	126	51	43	65	129	-
PM	65	79	32	ND	2717	+
VO	55	79	100	128	5481	+
HO	ND	115	54	38	110	-
BE	36	32	58	26	66	-
MS	165	ND	137	210	2667	+
Median value of IL-1	65	66	57	52		

Normal IgE value in adults < 150 units/ml. Italicized values are increased. ND: not done.
Clinical worsening: judged by symptoms score, blind evaluation of photographs and consumption of topical steroid.

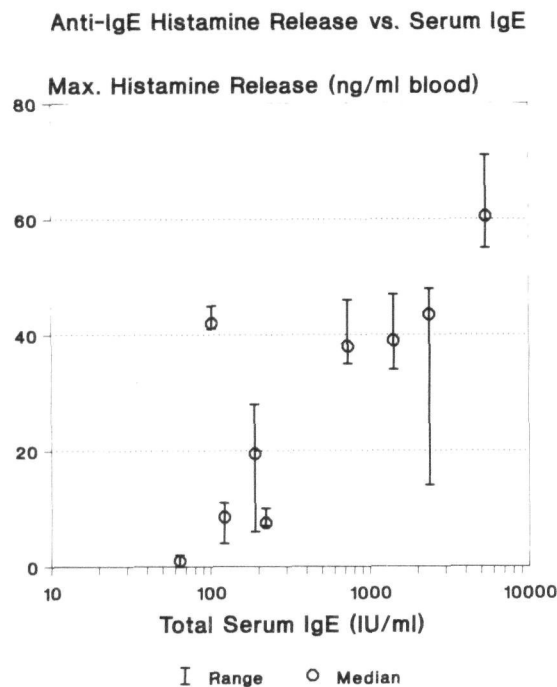


Fig. 1. Maximal histamine release to anti-IgE correlated with total serum IgE. The abscissa shows the median total serum IgE for the nine patients (log₁₀-scale). Maximal anti-IgE induced histamine release, median (O) and range (I) for the 4 weekly measurements are displayed on the ordinate axis.

significant histamine release was seen in the absence of anti-IgE. Activation of protein kinase C with TPA caused a minor histamine release, but had no significant dose-dependent effect on anti-IgE mediated histamine release.

The *in vitro* mitogen stimulation of mononuclear cells showed a reactivity comparable to a control group of 15 non-atopics studied simultaneously. The individual variations were large and there was no statistically significant change during the observation period (results not shown). We made a 70 % alcoholic extract of the two herbal drugs, evaporated the ethanol and prepared a solution of extract in tissue culture medium having 10 µg protein/0.1 ml. This was then added to mononuclear cells from three non-atopics in 10-fold dilutions. We did not see any mitogenic activity towards the lymphocytes compared with PHA stimulation in the same assay (culture period was 3 and 5 days). The interleukin-1 release from LPS stimulated Isopaque-Ficoll separated blood mononuclear cells was low (Table 1) and did not show any significant change.

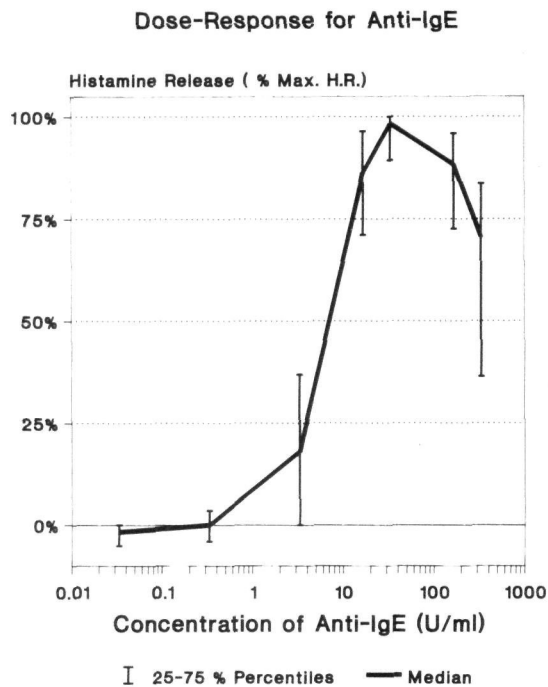


Fig. 2. The relative dose-response to anti-IgE in the 9 patients. Log₁₀ of anti-IgE concentrations are displayed along the abscissa. The histamine release is expressed as a percentage of the maximal anti-IgE induced release for each of the 36 dose-response experiments. Median and 25 % - 75 % quartiles are shown on the ordinate axis. Dose-responses were remarkably similar, irrespective of the variation in serum IgE and maximal histamine release (Fig. 1).

Soluble interleukin-2 receptors were measured in serum; and the results show that six of nine patients had increased levels of sIL2R compared with a group of nine controls (Fig. 4). The levels were stable during the observation period. Only one patient showed more than a 20 % increase which was, however, not accompanied by a clinical worsening of his disease. The level of sIL2R in serum did not correlate with IgE.

DISCUSSION

Herbal extracts or “drugs” are increasingly used by patients with chronic, non-curable diseases. A recent study in Norway has shown that approximately half of patients with atopic eczema use or have used such remedies (3). The drugs are mostly plant extracts, which are sold through pharmacies or drug stores. Theoretically, they may have a mitogenic, lectin-like effect on the human immune system. Preliminary evidence in-

icates that half of patients with atopic dermatitis have an increased absorption of large molecular weight polyethylene glycol compared with non-atopic patients. We have often experienced a clinical flare in patients with atopic dermatitis, who have tried herbal drugs. Whether this is due to an immune stimulatory capacity of the herbs, the simultaneous discontinuation of topical steroids, or a combination of both is not known.

We observed that five of nine patients experienced a flare of their symptoms. This was confirmed by patient, physician and photo score simultaneously. The severity of the flare was, however, moderate and could be controlled through increased consumption of the topical steroid. Due to ethical considerations our study design intended primarily to reveal if short-term clinical use of any herbal drug would increase the immune reactivity in the patients. We therefore did not include a pretreatment period with indifferent therapy or a wash-out period between the use of herbal extracts.

We have observed that patients with high IgE levels have a significantly higher release of histamine from basophils in washed blood, both

when stimulated with anti-IgE and with Con A. This seems to be in accordance with recent and earlier reports on the kinetics of the IgE-receptor-complex (9, 11, 12), but has not been found previously in patients with atopic dermatitis (10). Increased histamine releasability could be an explanation for the common flair reactions in AD patients with high IgE levels. A recent study of mast cells in the intestinal mucosa of AD patients demonstrated a significant correlation between the number of IgE-carrying mast cells and serum IgE levels, and also that these mast cells showed evidence of degranulation (5).

We did not observe any significant changes in *in vitro* activity following mitogen stimulation. A crude alcoholic extract of the herbal extracts did not induce cell proliferation of cells from non-atopic persons. Thus, our study does not support that herbal drugs can induce a lectin-like, mitogen effect in patients with AD.

The low interleukin-1 release from LPS-stimulated mononuclear cells confirms two previous reports (6, 15). We have observed that purified monocytes release significantly increased amounts of interleukin-1 in AD patients thereby creating a background for increased im-

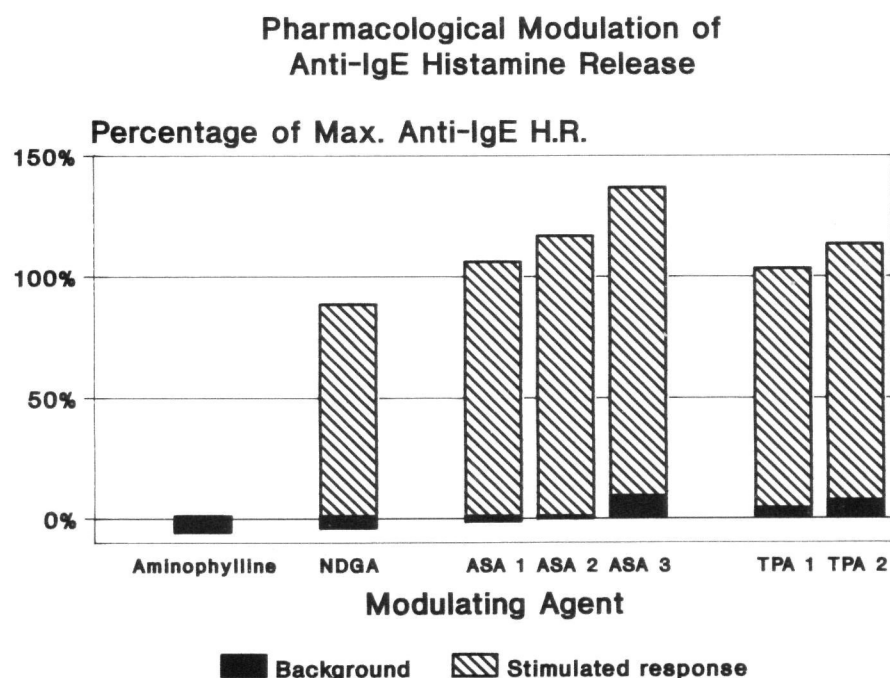


Fig. 3. The effect of preincubating washed blood cells for 10 min with various agents before adding anti-IgE. Preincubating compounds were aminophylline 2 mM, NDGA 10 μ M, ASA 55 μ M (1), 1.4 μ M (2) and 5.5 mM (3), TPA 10 ng/ml (1) and 50 ng/ml (2). Significant ($P < 0.05$) histamine release in absence of anti-IgE (background) was seen with ASA 5.5 mM and TPA 10 ng/ml and 50 ng/ml. Histamine release with anti-IgE was significantly depressed by aminophylline ($P < 0.01$), and significantly enhanced by ASA at 1.4 and 5.5 mM.

ATOPIC ECZEMA (N=9)

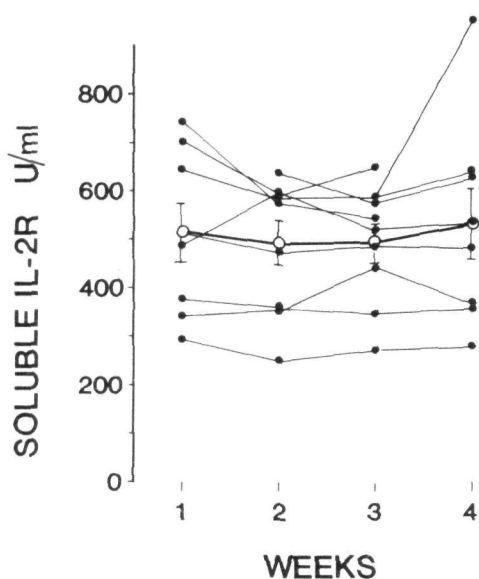


Fig. 4. The levels of soluble interleukin-2 receptors in serum of the patients (● — ●); (○ — ○) indicates the mean values. No significant changes were seen.

immune reactivity (20). We can confirm that soluble interleukin-2 receptor levels are increased in patients with atopic dermatitis (7). The ELISA assay measures a 55 kD light chain of the interleukin-2 receptor, which is released upon activation of T cells as a sign of increased activity in the immune system (16). The parameter is increased in rejection crises (1), during EBV infection (21), in active rheumatoid arthritis (18), and in patients with very active contact eczema, mycosis fungoides, parapsoriasis, and the red man syndrome (20).

In conclusion, our study shows that five of nine patients with AD had increased disease activity when taking herbal drugs recommended for "chronic skin problems". We did not observe any change in *in vitro* parameters.

It is a well-known observation that AD patients with high serum IgE levels tend to have a more severe AD (4). The significant correlation between an increased histamine release from blood basophils and serum IgE may be an important explanation of the many type I-like reactions experienced by these patients.

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REFERENCES

- Adams, D. H., Wang, L., Hubscher, S. G., Elias, E. & Neuberger, J. M.: Soluble interleukin-2 receptors in serum and bile of liver transplant recipients. *Lancet* *i*, 469-71, 1989.
- Jackson, P. G., Lessof, M. H., Baker, R. W. R., Ferrett, J. & MacDonald, D. M.: Intestinal permeability in patients with eczema and food allergy. *Lancet* *i*, 1285-86, 1981.
- Jensen, P.: Use of alternative medicine among atopic dermatitis and psoriasis patients. *Acta Derm. Venereol.* (Stockh) (in press).
- Juhlin, L., Johansson, S. G. O., Bennich, H., Hogman, C. & Thyresson, N.: Immunoglobulin E in dermatoses. Levels in atopic dermatitis and urticaria. *Arch. Dermatol.* *100*, 12-16, 1969.
- Kalimo, K., Lammintausta, K., Klemi, P., Leino, R., Panula, P. & Kalimo, H.: Mast cells and IgE in intestinal mucosa in adult atopic dermatitis patients. *Br. J. Dermatol.* *119*, 579-585, 1988.
- Kapp, A., Kirnbauer, R., Luger, T. A. & Schöpf, E.: Altered production of immuno-modulating cytokines in patients with atopic dermatitis. *Acta Derm. Venerol.* (Stockh) Suppl 144, 97-99, 1989.
- Kapp, A., Piskorski, A. & Schöpf, E.: Elevated levels of interleukin 2 receptor in sera of patients with atopic dermatitis and psoriasis. *Br. J. Dermatol.* *119*, 707-710, 1988.
- Larsen, C. G., Ternowitz, T., Larsen, F. G. & Thstrup-Pedersen, K.: Epidermis and lymphocyte interactions during a tuberculin skin reaction. I. Increased ETAF/IL-1-like activity, expression of tissue antigens and mixed skin-lymphocyte reactivity. *Arch. Dermatol. Res.* *280*, 80-89, 1988.
- Malveaux, F. J., Conroy, M. C., Adkinson, N. F., Jr. & Lichtenstein L.: IgE receptors on human basophils. *J. Clin. Invest.* *58*, 176-181, 1978.
- Marone, G., Giugliano, R., Lembo, G. & Ayala, F.: Human basophil releasability. II. Changes in basophil releasability in patients with atopic dermatitis. *J. Invest. Dermatol.* *87*, 19-23, 1986.
- Pruzandky, J. J. & Patterson, R.: Limiting concentrations of human basophil-bound IgE antibody required for histamine release. *Immunology* *64*, 307-310, 1988.
- Pruzandky, J. J. & Patterson, R.: Binding constants of IgE receptors on human blood basophils for IgE. *Immunology* *58*, 257-262, 1986.
- Rubin, L. A., Kurman, C. C., Fritz, M. E., et al.: Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol.* *135*, 3172-3177, 1985.
- Rubin, L. A., Kuman, C. C., Biddison, W. E., Goldman, N. D. & Nelson, L.: A monoclonal antibody, 7G7/B6 that binds to an epitope on the human IL-2 receptor distinct from that recognized by IL-2 or anti-Tac. *Hybridoma* *4*, 91-100, 1985.
- Räsänen, L., Lehto, M., Reunala, T., Jansen, C. & Leinikki, P.: Decreased monocyte production of inter-

- leukin-1 and impaired lymphocyte proliferation in atopic dermatitis. *Arch. Dermatol. Res.* 279, 215-218, 1987.
16. Rubin, L. A., Kurman, C. C., Fritz, M. E., et al.: Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J. Immunol.* 135, 3172-3177, 1985.
 17. Stahl Skov, P., Mosbech, H., Norn, S. & Weeke, B.: Sensitive glass microfibre-based histamine analysis for allergy testing in washed blood cells. *Allergy* 40, 213-218, 1985.
 18. Symons, J. A., Wood, N. C., Di Giovine, F. S. & Duff, G. W. Soluble IL-2 receptor in rheumatoid arthritis. Correlation with disease activity, IL-1 and IL-2 inhibition. *J. Immunol.* 141, 2612-2618, 1988.
 19. Thestrup-Pedersen, K., Cramers, M., Kongsholm, H. & Zachariae, H.: Bestatin therapy of patients with atopic dermatitis. *Acta Derm. Venerol. (Stockh)* 63, 549-552, 1983.
 20. Zachariae, C., Larsen, C. S., Larsen, C. G., Terno-witz, T., Kaltoft, K. & Thestrup-Pedersen, K.: Epider-mal-derived lymphokines and soluble IL2 receptor levels in mycosis fungoides. *Acta Derm. Venereol. (Stockh.)* 1990 (in press.)
 21. Tomkinson, B. E., Wagner, D. K., Nelson, D. L. & Sullivan, J. L.: Activated lymphocytes during acute Epstein-Barr virus infection. *J. Immunol.* 139, 3802-3807, 1987.

Address:
 Prof. *Kristian Thestrup-Pedersen*
 Department of Dermatology
 Marselisborg Hospital
 8000 Aarhus C
 Denmark

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