Acta Derm Venereol (Stockh) 1985: Suppl. 114: 48–54

Non-specific Immunotherapy and Specific Hyposensitization in Severe Atopic Dermatitis

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The mechanisms leading to the symptoms of allergic rhinitis and allergic asthma are today well characterized, while the pathophysiology of atopic dermatitis (AD) is still to a high degree obscure (1). There is, however, no doubt concerning the close association between the different atopic diseases and all may show dramatic increases in serum IgE and polyvalent sensitization against environmental allergens (2). Patients with AD also show other signs of lack of immunological balance such as an unusual susceptibility to skin infections (3, 4), low number of T-lymphocytes ant Tγ-lymphocytes in blood (5), low responsiveness in vitro to mitogens and antigens (6), a reduced antibody-dependent cellular toxicity of mononuclear cells and monocytes (7), reduced natural killer cell activity (8), and a defective nonspecific suppressor cell capacity (5). This lack of immunological balance together with the overall problems in treating patients with severe AD has lead us to try non-specific immune therapy as well as specific hyposensitization in adult patients with severe disease.

NON-SPECIFIC IMMUNOTHERAPY

Experimental non-specific immunotherapy was undertaken with transfer factor (TF), bestatin, and colchicine. All 3 agents have previously been reported to reduce IgE concentrations in serum.

TRANSFER FACTOR

Dialyzable leukocyte extract (TF) has been used with success in 2 children with severe AD (9, 10), while other workers (11) tried TF for weeks in adult patients in a double-blind cross-over study without effect.

We treated 10 patients, 5 men and 5 women, with TF or placebo for one year. TF was prepared from buffy-coat leukocytes from healthy blood donors. A detailed description of the production is given elsewhere (12). TF from 2×10^9 leukocytes was given intramuscularly every other week. As placebo we used sterile isotonic saline.

The same physician gave a clinical evaluation before treatment, each month during treatment, and one month after treatment. The extent and severity of the disease was quantitated and the use of topical steroids calculated monthly.

A number of in vivo and in vitro tests were performed both before, during, and after the treatment period. We used a Danish standard series of prick tests (12 extracts), RAST tests for dermatophagoides pteronyssinus, measurement of serum IgE, enumeration of T- and B-lymphocytes and subpopulations of T-lymphocytes, mitogen reactivity of lymphocytes, antibody-dependent cytotoxicity of monocytes, and isoproterenol stimulation of cAMP in monocytes.

The results of the clinical evaluation are given in Table IV. We found no evidence for an improvement in the dermatitis during therapy. Neither did we find any significant differ-

Table I. *Clinical response following hyposensitization together with responses in serum IgE*

Age/ sex	Treatment period	IgE (units) Before	Highest	After	Present (1983)	Clinical response	Present status (1983)
29/M	1977–1980	3 430	6 650	3 370	1 000	Improved	In remission
40/F	1976–1978 +since 1980	3 470	3 973	2 220	3 130	Improved	Slightly improved
24/F	1977–1978 +since 1979	7 860	13 000	1 900	2 300	Improved	Improved
29/F	1976–1978	10 900	27 800	14 150	4 870	Improved	In remission
36/F	1977–1980 +since 1980	6 370	7 500	3 580	2 900	Improved	Improved
62/F	1976–1980 +since 1980	11 300	22 900	3 600	11 900	Improved	Unchanged
48/F	1977–1980	1 790	3 440	2 190	–	Slightly improved	Slightly improved
37/F	1977–1980 +since 1980	2 550	7 200	2 340	3 680	Slightly improved	Improved
37/F	1977–1982	1 580	1 860	1 350	–	Slightly improved	Slightly improved
29/F	1976–1980	4 930	7 490	3 230	2 110	Unchanged	Improved
27/M	1977–1980	4 418	9 450	6 570	8 470	Unchanged	Improved
47/M	1978–1979	4 261	10 400	6 540	–	Worse	Not seen

ences between the 2 groups or any significant change in the immunological parameters during TF therapy. IgE values can be found in Table V. The total number of T-lymphocytes was significantly reduced in the patients compared with a control group, as was the monocyte-mediated cytotoxicity. The isoproterenol-induced cAMP increase in monocytes was low and did not change during the observation period (13).

The results of the investigations extend and confirm previous results of the use of TF in adult patients with AD. One criticism of our findings is that the skin reactivity in the TF treated group was not improved suggesting that our preparations were inactive. We can

Table II. *Urinary histamine per 24 hours in 7 atopics prior to and following 6 months of hyposensitization*

	Urinary histamine (μg/24 h) Before	After
4	37	37
6	156	63
7	75	60
8	34	118
9	47	120
10	28	69
11	51	28
Average ± SD	61±45	71±36

Table III. *Average consumption of Locoid® cream during hyposensitization, in 6 month periods*

	Period no. 1	2	3	4
No. of patients	9	9	9	7
Consumption (g)	355	205	95	103

only say that our method of preparation is widely used, and that in others of our studies transfer of skin reactivity has been found in skin test negative persons (14). We have previously shown that TF is able to induce a significant increase in cAMP in Tγ-lymphocytes (15). If Tγ-lymphocytes contain non-specific suppressor cells for IgE, then TF could possibly be of clinical value in AD. Observations by Jarisch et al. (16) have indicated that daily injections of TF in children with high levels of igE lead to a very pronounced reduction in the level of this antibody in serum. We have tried 2 i.u. per day of TF for 7 days in 4 adult patients with severe AD in order to see if such an intensive TF therapy could change the IgE concentration (13) but found no convincing effect.

Our conclusion is that TF, as given in the present study, is not able to influence the clinical course or immunoreactivity in adult patients with AD.

BESTATIN

Bestatin (2S, 3R)-3-amino-2-hydroxy-4-phenyl-butanoyl-L-leucine, a competitive inhibitor of aminopeptidase B and leucine aminopeptidase, augments various immune responses (17) and has recently been found to induce a transient reduction in IgE concentration in serum of healthy, non-atopic persons (18). We have therefore tried bestatin therapy in 10 adult patients with severe atopic dermatitis.

Table IV. *Results of clinical evaluations*

	Score severity and extent		Mean score per month	Ointment, used per month and range	The patients' own evaluation
	Before	After			
Placebo					
I	15	15	14	Hydrocortisoni butyras, 80 g (10–150 g)	Unchanged but less cold
II	8	6	8	Triamcinoloni acetonidum, 25 g (10–40 g)	Unchanged
III	2	1	1	Hydrocortisoni butyras, 12 g (10–25 g)	Better
IV	8	12	10	Hydrocortisoni, 30 g (10–55 g) hydrocortisoni butyras, 150 g (100–200 g)	Unchanged
V	15	15	15	Hydrocortisoni butyras, 230 g (175–300 g)	Unchanged
Average	9.6	9.8	9.6		
TF					
VIII	6	12	9	Hydrocortisoni butyras, 55 g (25–100 g)	Unchanged
IX	3	4	4	Hydrocortisonum, 49 g (20–75 g)	Unchanged
X	9	6	7	Fluocinoloni acetonidum 35 g (25–50 g)	Less prurigo
XI	6	6	7	Hydrocortisoni butyras, 65 g (20–100 g)	Better
XII	9	9	7	Hydrocortisoni butyras, 220 g (120–130 g)	Unchanged
Average	6.6	7.4	6.8		

Bestatin was gives as tablets 20 mg twice daily for three months. The study was conducted over a one year period with 2 or 3 participants every fourth month in order to avoid a bias from seasonal variation. The trial was "open" and the clinical evaluation was done monthly by one of us. Apart from bestatin the patients received topical steroids. All participants completed the trial without any side-effects.

The clinical evaluation was done by giving points for the disease activity in 5 different regions, the highest possible score being 20. Five of the patients participated in an immunological monitoring before and during the treatment period.

The clinical evaluation showed that bestatin was not able to change the course of the dermatitis, and no statistically significant changes occurred in the laboratory data (Table VI). The individual serum IgE concentrations are shown in Table VII.

Our conclusion is that bestatin seems to be without value in the therapy of patients with AD.

COLCHICINE

Colchicine has for many years been used in gout. The drug inhibits chemotaxis of polymorphonuclear leukocytes (19), blocks leukocyte adhesiveness (20), and stabilizes lysosomal membranes (21). Suppression of inflammatory responses by colchicine has also been shown in animal experiments, and the DNCB-induced irritant dermatitis has been markedly inhibited (22). Within recent years colchicine has been tried in cutaneous vascular diseases (23) as well as in familial Mediterranean fever (24). In 1982 Ilfeld & Kuperman (25) reported of the correction of a deficient Con-A activated suppressor cell function in 4 patients with the latter disease by colchicine. This lead us to try the drug in 10 patients with severe atopic dermatitis and high values of serum IgE.

Colchicine was given as tablets 1 mg twice daily for 4 weeks. All patients were treated simultaneously during the spring (March/April) 1983.

Also this trial was performed as an open study and the clinical evaluation was done after 2 and 4 weeks by the same physician. All patients were allowed to continue with a topical treatment, a group II steroid. Besides mild diarrhoea in 2 patients no side effects were

Table V. *IgE in serum, IU/ml*

No.	Before	3 months	6 months	12 months
Placebo				
I	897	867	1 460	1 560
II	2 560	3 380	2 400	1 610
III	2 080	1 910	1 950	1 690
IV	3 530	3 180	2 260	1 850
VI	8 880	16 100	11 100	11 700
Mean	3 589	5 087	3834	3 682
TF				
VIII	633	1 160	689	1 230
IX	2 240	2 130	2 660	1 790
X	2 020	1 520	1 430	1 790
XI	1 840	4 550	4 490	1 950
XII	4 250	4 330	3 660	4 750
Mean	2 197	2 738	2 586	2 302

Table VI. *Bestatin therapy of patients with atopic dermatitis. Clinical score*

Patients	Age	0 months	1 month	2 months	3 months
Woman	24	8	10	12	12
Woman	28	8	10	8	7
Woman	35	16	9	6	13
Woman	40	15	18	14	16
Woman	38	18	18	17	15
Woman	35	6	5	3	5
Woman	35	13	9	6	6
Woman	61	16	14	13	12
Woman	32	15	13	17	14
Man	27	9	9	6	9
Median value	35	14	10	10	12

recorded. The clinical scores were the same as in the bestatin evaluation. Serum IgE was investigated before and at each control visit. In seven patients polymorphonuclear leukocyte chemotaxis was also studied by the method of Gallin et al. (26) using N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Serva, Heidelberg) as chemotaxin.

Although polymorphonuclear leukocyte chemotaxis was reduced in all 7 patients establishing the activity of the drug, no patients improved clinically and serum IgE did not change during the study. The laboratory data can be seen on Tables VIII and IX. Colchicine was found to be without clinical value in AD.

HYPOSENSITIZATION

How hyposensitization works in respiratory atopic disease is not fully understood (27), but it is a common finding that following an initial increase in serum IgE, this parameter later decreases. The following mechanisms have been discussed: Blocking antibodies, induction of suppressor T-cells, the possible formation of antiidiotype antibodies as well as an altered susceptibility of mediator-secreting cells. Due to conflicting data on the results of hyposensitization in AD (1) we felt it reasonable to re-study this approach in patients with

Table VII. *Bestatin therapy of patients with atopic dermatitis. Serum IgE concentrations (IU per ml)*

Patients	Age	0 mo.	1 mo.	2 mo.	3 mo.
Woman	24	2 110	1 890	1 990	3 970
Woman	28	3 430	1 990	1 860	2 090
Woman	35	993	1 130	1 190	1 570
Woman	40	4 720	4 900	4 920	4 680
Woman	38	7 620	7 670	5 290	6 590
Woman	35	4 700	4 160	3 180	3 480
Woman	35	1 690	1 860	1 940	1 740
Woman	61	9 160	9 000	9 450	10 900
Woman	32	12 900	12 000	12 600	16 000
Man	27	5 960	5 600	4 990	5 075
Median value		4 710	4 480	4 050	4 325

Table VIII. *Chemotactic index in atopics before and after two weeks Colchicine 2 mg daily*

	CI	
Pat. no.	Before	After
1	4.4	1.2
2	3.4	1.6
3	3.5	1.4
4	3.4	1.7
5	2.2	1.3
6	2.6	1.9
7	2.6	1.6

Table IX. *Serum IgE in 9 atopics prior to and after two weeks Colchicine 2 mg daily*

	IgE (units)	
Pat. no.	Before	After
1	2 740	3 430
2	9 370	7 780
3	17 300	17 160
4	1 320	1 640
5	24 000	20 000
6	3 910	1 990
7	7 160	6 400
8	11 100	13 300
9	15 000	11 400

severe disease using an allergen which is contantly to be found in the sensitized patient's environment.

Twelve adult patients sensitive to house dust mites were hyposensitized. Three also suffered from asthma, 3 from rhinitis, and 2 patients displayed all three main atopic diseases. The major problem was, however, in all patients AD, and this was the only purpose for treatment.

Injections were given in increasing dosages with intervals increased from one to eight weeks. All patients were allowed to use a medium-strength topical steroid (Locoid®) according to their needs. The cream was delivered by us and the amounts used were measured in 9 of the patients.

Six patients improved (Table I), 3 patients showed slight improvement, while 2 patients were unchanged and one patient got worse after the observation period which lasted from 1½ to 5 years. Most but not all patients followed the pattern of an increase in serum IgE, followed by a decrease. There was similar but less pronounced changes in specific antibody activity as measured by the radio-allergo-sorbent-test (RAST). However, no significant change in RAST class was observed. Two patients were reduced from class 4 to class 3, and 2 from class 2 to class 3 or 4. Urinary histamie was studied in 7 patients (Table II) and was found unchanged following 6 months of treatment and generally to be within normal values. The consumption of topical steroid was found to be reduced during treatment (Table III).

When evaluated from 4 to 7 years after initiation of therapy 7 of 11 declared that their present condition was significantly better than prior to treatment. Five still received hyposensitization.

It is our present opinion based upon our investigation as well as on more recent reports in the literature (28, 29) that it may be worth-while to try hyposensitization in cases of severe AD.

COMMENTS

In conclusion, none of our three experimental trials using non-specific immunotherapy can be considered promising in the treatment of AD. Specific hyposensitization with extracts of house dust mite, an allergen constantly found in the sensitized patient's environment, however, in general left the patients in a better condition than prior to the study.

REFERENCES

1. Rajka G. Atopic dermatitis. London: W. B. Saunders, 1975.
2. Johansson S, Bennich H, Berg T. The clinical significance of IgE. In: Schwartz R, ed. Progress in clinical immunology. New York: Grune & Stratton. 1972: 157–181.
3. Copeman P. Wallace H. Eczema vaccinatum. Br Med J 1964; II: 906–908.
4. Jones H, Reinhardt J, Rinaldi M. A clinical, mycological and immunological survey for dermatophytosis. Arch Dermatol 1973; 108: 61–65.
5. Jensen J, Cramers M, Thestrup-Pedersen K. Subpopulations of T-lymphocytes and non-specific suppressor cell activity in patients with atopic dermatitis. Clin Exp Immunol 1981; 45: 118–123.
6. Thestrup-Pedersen K, Ellegaard J, Thulin H, Zachariae H. Mitogen and PPD responsiveness in severe atopic dermatitis. Clin Exp Immunol 1977; 27: 118–126.
7. Kragballe K, Herlin T, Jensen J. Impaired monocyte cytotoxicity in atopic dermatitis. Arch Dermatol Res 1980; 269: 21–29.
8. Jensen J, Jørgensen A. Thestrup-Pedersen K. Modulation of natural killer cell activity in patients with atopic dermatitis. J Invest Dermatol 1984; 82: 30.
9. Alonso A. Transfer factor in atopic dermatitis. Lancet 1976; I: 1352.
10. Strannegaard I-L, Hansson L, Mobacken H, Strannegaard O. Transfer factor in severe atopic disease. Lancet 1975; II: 702.
11. Hovmark A, Ekre H. Failure of transfer factor therapy in atopic dermatitis. Acta Derm Venereol (Stockh) 1978; 58: 497–500.
12. Thestrup-Pedersen K, Grunnet E, Zachariae H. Transfer factor in mycosis fungoides. A double-blind study. Acta Derm Venereol (Stockh) 1982; 62: 47–55.
13. Cramers M, Jensen J, Kragballe K, Herlin T, Zachariae H, Thestrup-Pedersen K. Transfer factor in atopic dermatitis. Dermatologica 1982; 164: 364–378.
14. Thestrup-Pedersen K, Thulin H, Zachariae H. Transfer factor applied to intensify the cell-mediated immunological reactions against mycobacterium avium. Acta Allergol 1974; 29: 101–116.
15. Herlin T, Jensen J, Thestrup-Pedersen K, Zachariae H. Dialyzable leukocyte extract stimulates CMP in Tγ-lymphocytes. Allergy 1981; 36: 337–343.
16. Jarisch R, Eibl M, Sandor I, Boltz A. Influence of dialyzable transfer factor on IgE concentrations in patients with atopic dermatitis. Allergy 1981; 36: 99–105.
17. Bruley-Rosset M, Florentin I, Kiger N, Schultz J, Mathé G. Restoration of impaired immune functions of aged animals by chronic bestatin treatment. Immunology 1979; 38: 75–83.
18. Blomgren H, Forsgren M, Nordberg R, von Stedingk L-V, Wasserman J. Influence of bestatin, a new immunomodulator on Ig secretion by human lymphocytes in vitro and in vivo. Int Arch Allergy Appl Immunol 1981; 64: 338–344.
19. Caner J. Colchicine inhibition of chemotaxis. Arthritis Rheum 1965; 8: 757–760.
20. Penny R, Galton D, Scott J. Studies on neutrophil function. Br J Haematol 1966; 12: 623–632.
21. Rajan K. Lysozomes and gout. Nature 1969; 210: 959–960.
22. Miyachi Y, Takigawa M, Imamura S. Suppression of DNCB-induced irritant dermatitis by colchicine. Acta Derm Venereol (Stockh) 1981; 61: 307–311.
23. Hazen O, Michel B. Management of necrotizing vasculit with colchicine. Arch Dermatol 1979; 115: 1303–1306.
24. Dinarello C, Wolff S, Goldfinger. Colchicine therapy for familial Mediterranean fever. N Engl J Med 1974; 291: 934–937.
25. Ilfeld D, Kuperman O. Correction of a suppressor cell deficiency in four patients with familial Mediterranean fever by in vitro and in vivo colchicine. Clin Exp Immunol 1982; 50: 99–106.
26. Gallin J, Clark RA, Kimball H. R. Granulocytochemotaxis, an improved in vitro assay employing 51 Cr labelled granulocytes. J Immunol 1973; 110: 233–240.
27. Freedman S. Asthma and allergic rhinitis, clinical aspects. In: Freedman S, Gold PH, eds. Clinical immunology. Hagerstown: Harper & Row, 1976: 131–168.
28. Kaufman H, Roth H. Hyposensitization with alum precipitated extracts in atopic dermatitis—a placebo-controlled study. Ann Allergy 1974; 32: 321–327.
29. Ring J. Successful hyposensitization treatment in atopic dermatitis: results of a trial in monozygotic twins. Br J Dermatol 1982; 107: 597–602.

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