

Influence of Dialysable Transfer Factor on IgE Concentrations in Patients with Atopic Dermatitis

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Dialysable transfer factor (TF) was given in 10 paediatric patients with severe atopic dermatitis (AD). Ten patients with AD, matched for age and severity of disease, served as controls.

Prior to the therapy with TF and at weekly intervals thereafter, T- and B-cells in the blood, PHA-stimulation, total IgE and specific IgE antibodies to inhalant and food antigens were determined. Therapy with TF was followed by IgE depression in 8/10 patients and was most pronounced in three patients with initially high levels. Some decrease of IgE levels was seen in four controls also, none of them, however, fell to normal levels as was seen in two of the treated patients.

Specific IgE levels decreased slightly, but always remained within the pathological range. T-cell counts in the blood increased in 2/10 cases as well as PHA-stimulation. B-cell counts remained within normal limits. Clinical improvement was seen in one patient, five improved slightly and four remained unchanged.

Our results indicate, that transfer factor can lower total IgE levels in cases with atopic dermatitis. The effect is most marked in patients with high total IgE levels. Skin involvement, however, does not closely follow *in vitro* findings.

Key words: IgE; T-cells; transfer factor; viral infection.

Accepted for publication 7 August 1980

Presented in part at the Annual Meeting of the Swiss Society of Allergology and Immunology, March 22th/23rd 1979, Zürich.

Immunological studies relating to atopic dermatitis reveal two characteristic findings: raised IgE levels (15, 19, 28) and evidence of dysfunction in cell-mediated immunity (8, 9, 21, 25). Clinical correlates to elevated IgE levels are the frequency of coexisting allergic respiratory diseases, and to T-cell dysfunction the increased susceptibility to viral infections.

Studies by Tada and his colleagues, in rats, demonstrated the existence of suppressor T-cells exerting a negative regulatory control over the production of IgE antibodies in this species (26). Similar findings were also published by Katz and co-workers (12). Enhanced

IgE-antibody response induced by irradiation or cyclophosphamide could be lowered by passive transfer of thymocytes or splenic T-cells.

This reversibility of enhancing or suppressing IgE-antibody production suggests that suppressor T-cells are involved in non-specific control of IgE production.

During recent years TF has been tried as a therapeutic agent in patients with immunodeficiency disorders such as Wiskott Aldrich syndrome (22) and chronic candidiasis (3). Successful use of TF therapy has been reported in two children with severe atopic dermatitis (2, 24). A favourable result was seen in one out of three

patients by Thulin et al. (27) and no response to TF therapy was reported in six patients by Hovmark & Ekre (10).

We therefore investigated the influence of dialysable transfer factor in 10 paediatric patients with atopic dermatitis. The aim of the study was to clarify whether transfer factor has an effect on the level of IgE-antibodies and T-cell function, as well as on the clinical state.

MATERIAL AND METHODS

Patient selection

Ten patients (two girls and eight boys aged from 3 to 16 years, average 9.6 years) with clinical evidence of AD for some years were admitted to the hospital for 3 weeks. Eczematous lesions were present on at least three typical regions (flexure of the extremities and the neck, and characteristic pigmentation in the periorbital region). In addition, elevated total IgE levels and specific IgE-antibodies to food and/or inhalant antigens were detected at previous testing. Ten patients with atopic dermatitis, matched for age and severity of disease, served as controls.

Total IgE. Determinations were done with the Phadebas PRIST® method according to the instructions. Levels above 100 u/ml were considered pathological.

Specific IgE-antibody. Determinations of specific IgE-antibodies against inhalant and food antigens were measured by Phadebas RAST®. The tests were run, and results were evaluated according to standard techniques. All sera were tested for specific IgE-antibodies against house dust, house dust mite, cat-, dog- and horse dander, the moulds *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Mucor racemosus* and *Alternaria tenuis*, grass pollen, birch pollen and egg, milk and fish. The results were given in RAST classes (0–4): RAST classes 2–4 were considered positive. The reproducibility of the tests in our hands was within one RAST class.

T-cell counts. E-rosette assays were carried out according to the method of Jondal et al. (11). Briefly: mononuclear cells were obtained by the Ficoll-Hypaque isolation, 0.1 ml of a 0.5% sheep erythrocyte suspension was added to 5×10^5 mononuclear cells in the presence of 0.02 ml of heat-inactivated human serum absorbed with sheep erythrocytes. Cells were centrifuged at 130 g, placed in a refrigerator overnight and read the next morning. Control values by this method vary between 60% and 80% (mean value of 50 controls: $66\% \pm 14\%$). Values below 50% were considered as pathological.

B-cell counts. B-cells were determined by detection of membrane-bound immunoglobulin with standard techniques and with commercially available conjugated antisera (HYLAND: goat anti-human immunoglobulin) Cells with membrane-bound immunoglobulins ranged between 10% and 20%. (1)

PHA-stimulation. Microcultures were used, and whole blood technique was carried out. PHA (PHA P, DIFCO Lab.) was used in dilutions of 1:25, 1:125, 1:625. The optimal stimulating dose, with a few exceptions, was 1:125. Tritiated thymidine was added after 56 h; tests were harvested after 72 h of culture and the uptake of tritiated thymidine measured and expressed in c.p.m. A healthy person was tested parallel at each occasion. Results were considered to be depressed when maximal stimulation of a patient was below 33% of the day's "control".

Dialysable transfer factor.* The TF donors were healthy blood donors with no obvious history of atopy. Material was pooled from over 500 donors and one batch was used throughout the study. The preparation followed the methodology of Grob et al. (7). This preparation was shown to be active in the sense that new delayed hypersensitivity reactions (tuberculin, candida) developed in negative recipients. It was also demonstrated, that patients with secondary T-cell deficiency (severe viral infections) with low numbers of sheep erythrocyte rosetting cells

* Kindly supplied by Immuno AG, Vienna.

Tabel 1

Case No.	Clinical state after TF therapy		Total IgE (PRIST)	T-cells %	B-cells %	PHA ($\times 10^3$)
1	A		7000	n.d.	n.d.	68 **
	B		900	52	12	76
	C	++	400	n.d.	n.d.	56
	D		250	45	13	101
2	A		6100	23	24	34 **
	B		800	20	10	46
	C	+	350	31	30	n.d.
	D		90	47	14	86
3*	A		5200	48	12	66 **
	B		90	71	8	66
	C	=	100	58	10	58
	D	(pyodermas)	2400 +	27	8	68
4	A		2600	59	4	164
	B		800	60	12	n.d.
	C	+	450	76	3	93
	D	(pyodermas)	550	78	3	135
5	A		1600	n.d.	14	240
	B		1500	50	8	189
	C	+	1100	52	8	n.d.
	D		900	50	11	110
6	A		1500	n.d.	4	167
	B		1150	61	6	83
	C	+	660	54	7	n.d.
	D		1200	66	7	103
7	A		900	62	10	142
	B		800	48	16	124
	C	=	n.d.	n.d.	n.d.	n.d.
	D	(pyodermas)	450	52	12	146
8*	A		600	17	16	45 **
	B		120	39	26	67
	C	+	190	40	16	45
	D	(pyodermas)	8400	62	10	64
9	A		550	57	24	70
	B		1700	42	12	88
	C	-	n.d.	56	9	53
	D	(erythroderma)	900	n.d.	9	280
10	A		90	57	8	153
	B		20	53	8	116
	C	=	70	38	12	101
	D		40	54	10	134

A = prior therapy
 B = 1 week after
 C = 2 weeks after
 D = 3 weeks after

* = viral infection
 ** = PHA response depressed
 (below 33% of the day's controls)

++ = improved
 + = slightly improved
 = = unchanged
 - = worse

(T-cells) showed an increase of T-cells to low normal values after treatment (unpublished observations).

Transfer factor therapy. TF was given in a dosage of 1 TF unit (prepared from 2×10^9 leucocytes)/10 kg body weight/week. The lyophilised TF was dissolved in 5.0 ml distilled water and 2.5 ml were administered subcutaneously at 2-h intervals.

A maximum of 1 TF (= six injections every 2 h) was given a day. Therapy was started (first day) by giving 0.1 ml intradermally and then 0.1; 1 and 2 ml subcutaneously at hourly intervals. After which 2.5 ml were given at 2-h intervals until the dosage of 1 TF/day was reached.

Topical treatment. An emollient (Ultrabas®) was used for topical treatment. In three cases with no clinical improvement steroids were applied topically during the last week of treatment.

Statistical evaluations were done by the Fisher's t test.

RESULTS

Total IgE

Total IgE levels in the 20 AD patients are summarized in Table 2. In the 10 patients treated, six had pretreatment IgE levels above 1000 u/ml, three between 100 and 1000 u/ml and one below 100 u/ml. In the untreated group four patients had values above 1000 u/ml and six between 100 and 1000 u/ml.

Depression of total IgE levels during the course of TF therapy was most pronounced in patients with high initial levels (cases 1, 2, 3). In these patients normal values were reached after TF therapy. Decrease of IgE levels was also seen in cases 4, 5, 6, 7 and 8, although results were not so distinct. The rise of IgE levels in cases 3 and 8 occurred during a severe influenza which was followed by an acute exacerbation of AD. Case 9 did not respond to therapy at all: IgE level rose, and skin lesions developed into an

Table 2

Total IgE levels in patients ($n = 10$) with atopic dermatitis treated with transfer factor measured at weekly intervals compared with untreated matched controls ($n = 10$)

TF treated		Prior therapy	1 week	2 weeks	3 weeks thereafter
group	Case No.				
	1	7000	900	400	250
	2	6100	800	350	90
	3	5200	90	100	2400*
	4	2600	800	450	550
	5	1600	1500	1100	900
	6	1500	1150	650	1200
	7	900	800	450	—
	8	600	120	190	8400*
	9	550	1700	—	900
	10	90	20	70	40

* = viral infection

Untreated group	First blood sample	3 weeks thereafter
Control No.		
1	5000	1300
2	1300	1750
3	1200	1400
4	1050	2300
5	950	300
6	950	1050
7	700	450
8	500	180
9	300	400
10	200	350

erythroderma. Case 10 had normal IgE levels prior to and after therapy and did not improve.

A slight decrease of total IgE could be seen in 3/10 of the control patients (Table 1). Statistical analysis of the treated and the untreated group, however, showed no significance $0.05 < P < 0.10$.

Specific IgE antibodies

Antibodies against seven inhalant and three food antigens were tested in each patient. A decrease of more than one RAST class was seen in 23 out of 110 tested allergens in the treated group compared with 16/110 in the untreated

group. An increase was seen in 1/110 in the treated group compared with 9/110 in the untreated group. The difference is statistically significant ($P < 0.02$) using Fisher's *t* test. In no case, however, did specific IgE antibodies fall to normal levels.

T-cell counts

E-rosetting lymphocytes were below 50% in three patients from the treated group and in five of the controls. The E-rosetting population increased in all three patients during therapy with transfer factor.

B-cell counts

In all cases B-cell counts remained within normal limits (Table 1).

PHA-stimulation

PHA-stimulation was below 33% of control values in patients No. 1, 2, 3, 8. Values increased slightly during treatment (Table 1).

Clinical course

Skin lesions improved in patient No. 1, slightly improved in patients No. 2, 4, 5, 6 and 8, were unchanged in patients No. 3, 7 and 10, and were worse in patient No. 9 (Table 1).

It should be noted, that topical application of steroids was necessary in patients No. 3, 7 and 8.

Side effects

Apart from local pain, which occurred briefly after injection, no side effects were seen. The full therapy schedule could be given in all cases.

DISCUSSION

Publications on transfer factor therapy in atopic dermatitis claim varying results. Good results (2, 13, 16, 18, 24) are contrasted by those in which no improvement was reported (10, 26). Unfortunately, these papers deal with the findings in one to three patients. Thulin and co-workers

(27) found no improvement *in vivo* and *in vitro* in their patients. These were adults, however. In addition the dosage used was much lower than that used in our study.

Stimulated by the findings of Tada (26) and Katz (12) in animals we evaluated the effect of TF administration on the E-rosette-forming lymphocyte population, PHA-stimulation and concentration of IgE in serum, as well as on the clinical state of patients with atopic dermatitis.

It is known that TF affects T-cells and increases their capacity to form rosettes with sheep erythrocytes and to respond to mitogens such as PHA. It is more likely that instead of transferring reactions of donor specificity TF enables lymphocytes of the recipient to respond to antigens encountered previously (3, 4, 12, 14). Two patients out of 10 (cases 2 and 8 in our series) had markedly reduced numbers of E-rosetting lymphocytes. Their cells also responded poorly to PHA. Decreased responsiveness to PHA was seen in three additional patients (cases 2, 3, 9) whose E-rosetting cell population was borderline between 50 and 60%. Several T-cell subpopulations are known to have regulatory functions in antibody production. The importance of these cells is also established for regulating the IgE response. As previously shown certain aspects of this function are class specific: suppressor T-cells control IgE levels (12, 26). Defects in their function (achieved experimentally by irradiation or cyclophosphamide) will result in elevated IgE levels, as has been shown previously in animals (9, 21). The most striking effect of TF in our study was the decrease of total IgE in eight out of 10 patients, most impressive in those patients who had the highest levels prior to therapy (Table 1).

Total IgE levels in 10 untreated control patients with atopic dermatitis, also showed a decrease in some patients, the differences between treated and untreated groups, however, was not significant $0.05 < P < 0.10$. It is worth noting that a marked drop of total IgE was seen in patients No. 1, 2 and 3 leading to a level of 2–3% of the pretreatment value. Since the half-life of IgE antibody is said to be 2 days (21), this would mean that in these patients a total stop of total IgE production has been reached. This marked

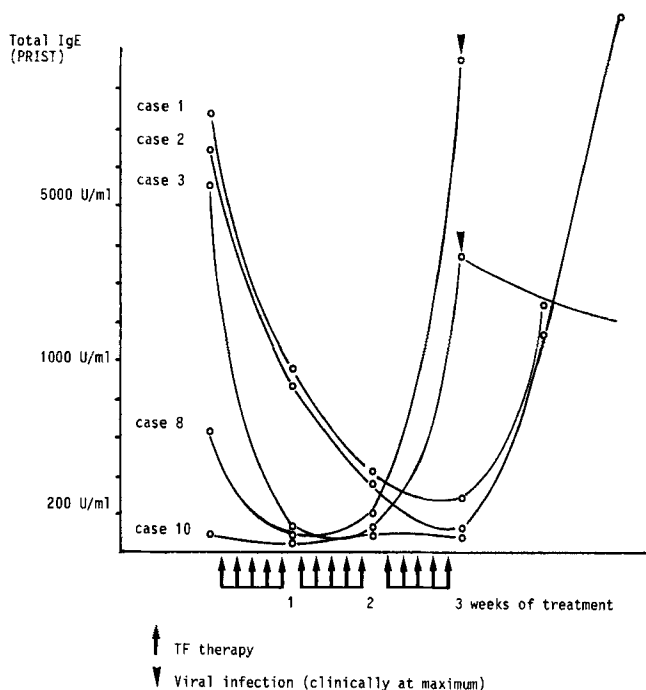


Fig. 1. Total IgE levels during and after TF therapy: Influence of viral infections and termination of TF therapy on total IgE levels.

drop in total IgE in three patients treated with TF, was not seen in any of our control patients. It appears likely that these findings can be related to TF-treatment.

Specific IgE antibodies remained unchanged in the control group. In the treated group, even though there was a significant ($P < 0.02$) decline, baseline (negative) values were never reached.

An increase of the T-cell population in peripheral blood associated with decrease of high total IgE level has also been found in patients with *Mycosis fungoides* following TF treatment (29). The decrease of total IgE in our patients is also in accordance with experimental results obtained in rats (12, 25). The decrease in IgE is likely to be due to increased suppression.

Even the reversibility of IgE antibody suppression can be demonstrated by our findings. Measuring total IgE levels after termination of therapy we saw a rise of IgE levels in eight out of 10 patients (cases 1 and 2 in Fig. 1). Patient No.

10 should be excluded since *in vitro* findings were normal before therapy except for slightly elevated IgE antibodies to egg and milk.

An experiment by nature (6) can be seen in cases 3 and 8 in whom a severe viral infection (influenza) was followed by a marked increase of total IgE level. While the patients improved clinically and dermatologically the total IgE levels fell again (Fig. 1).

In contrast to our *in vitro* findings, impressive changes in the clinical state were not seen. An unexpected side effect was the increased frequency of pyoderma in four patients, which lasted for several weeks and was seen less often in the other atopic dermatitis patients.

In our study the lack of correlation between clinical course and laboratory data illustrates that atopic dermatitis is influenced by several factors, one of which could be an altered cyclic nucleotide system (5, 18). We therefore think that therapy of AD has to take into consideration known altered immunological as well as phar-

macological findings to influence both clinical symptomatology and immunological reactivity.

ACKNOWLEDGEMENTS

We wish to thank Mrs Irene Matejovsky, Mrs Emmy Zambo, Mrs Erika Tenczar and Mrs Elisabeth Stifter for skilful assistance.

This study was supported by a grant from the Fonds zur Förderung der wissenschaftlichen Forschung (No. 4036), Vienna, Austria.

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