Acta Dermatovener (Stockholm) 58: 497–500, 1978

FAILURE OF TRANSFER FACTOR THERAPY IN ATOPIC DERMATITIS

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Abstract. A controlled clinical study was conducted on 6 patients with atopic dermatitis to assess the efficacy of transfer factor. After the code was broken the 3 patients treated with placebo preparation were treated with transfer factor for a further period of 10 weeks. No definite therapeutic effects could be demonstrated. The immunological in vivo and in vitro tests failed to reveal any effects except for a change to positive in the tuberculin skin test in those patients who had previously been skin test negative. The treatment had to be discontinued in one patient due to a suspected allergic reaction against transfer factor.

Key words: Hypersensitivity, delayed

During recent years Transfer Factor (TF) has been used as a therapeutic and immunorestorant agent in patients with immunodeficiency diseases such as Wiskott-Aldrich's syndrome (13), and chronic candidiasis (2). Patients with atopic dermatitis (AD) have both in vivo and in vitro signs of depressed cell-mediated immunity (7, 15) and immunological disturbances still remain when the eczema is almost healed, indicating that there may be a basic primary abnormality in the immune system (8). They also often have increased serum levels of IgE and it has been demonstrated in rats (16) that a defective suppressor function of the T lymphocytes may cause increased IgE production. As TF has been reported to restore depressed cell-mediated immunity in immunodeficiency diseases, it might be of interest to study the immunological and clinical effects of TF therapy in patients with AD.

MATERIAL AND METHODS

Patient selection

Four women and 2 men were enrolled in the study. All patients were over 18 years old and suffered from severe atopic dermatitis. None of the patients had severe asthma or rhinitis. During the periods of investigation the patients used hydrocortisone creams locally, antihistamine tablets when they suffered from severe pruritus, and antibiotics when they had secondary infection of the skin.

Transfer Factor preparation

The preparation of TF was performed essentially as described by Basten et al. (2) and will be reported in detail elsewhere. Briefly, buffy coats from normal blood donors, all tested and found negative for HB_sAg (Hepatitis B surface antigen) by radioimmunoassay, were pooled and the mononuclear leukocytes purified by centrifugation over a Ficoll-hypaque gradient (Lymphoprep, Nyegaard, Norway) (5). The cells were frozen and thawed ten times in distilled water and ultrafiltered in a Sartorius system (SM 16525 apparatus; SM 12136 membranes). After passing through a 0.22 μm Millipore filter the ultrafiltrate was ampouled, lyophilized and stored at $-20°C$ until used. The content in each ampoule, one unit of TF, corresponds to 5×10^8 E-rosetting lymphocytes ($7\text{–}8 \times 10^8$ leukocytes). The peptide content was estimated by using fluorescamine (19) (Fluram; Roche, Basle, Switzerland) with bovine insulin (Sigma, St. Louis, USA) as a standard. The ability of TF to augment the response to pokeweed mitogen (PWM) and PPD was tested in the lymphocyte transformation test (LTT) using lymphocytes from healthy adults.

Transfer Factor therapy

One unit of TF dissolved in 4 ml saline solution was given i.m. once a week for 10 weeks. The first part of the study (Januari–April 1977) was performed as a double-blind investigation in which 3 patients were given TF and 3 patients injections of saline solution. The second part (September–December 1977) was done as an open study where the patients who previously had received placebo were treated with TF.

Clinical evaluation

Clinical evaluations were performed before, during and after the periods of treatment. The extent of active dermatitis (percentage of total body surface), pruritus and secondary infections were recorded. Photographs of involved skin areas were taken before and after the test periods.

Immunological evaluation

Test for immunological evaluation were performed before and after the periods of treatment. Intracutaneous (i.c.) tests were carried out with 0,1 ml PPD-tuberculin 2 T.U. (Statens seruminstitut, Copenhagen, Denmark), 0.1 ml

Table I. *Results of TF and placebo treatment in patients with atopic dermatitis*

+++=active dermatitis on >75 % of the body surface. ++=active dermatitis on 25–75 % of the body surface. +=active dermatitis 5–25 % of the body surface

	Treatment	Patient	Activity of the dermatitis	IgE/S U/ml	Tuberculin skin test	LTT with PPD (ratios)	T-cells in peripheral blood×10^9/l
First test period	TF	LE before	++	7 400	Neg	1.3	1.0
		LE after	++	4 500	Pos (7×8 mm)	1.9	1.1
		SL before	+	9 300	Pos (16×16 mm)	3.5	0.8
		SL after	+	11 500	Pos (15×15 mm)	13.3	1.8
		KH before	++	9 000	Neg	1.6	1.3
		KH after	++	11 900	Pos (9×9 mm)	2.2	1.4
	Placebo	UK before	+	600	Neg	2.7	0.7
		UK after	++	1 100	Neg	–	1.1
		UM before	++	11 500	Pos (15×20 mm)	4.9	1.3
		UM after	++	4 100	Pos (12×12 mm)	4.1	0.9
		KM before	++	5 800	Neg	7.8	1.1
		KM after	+	3 100	Neg	3.5	0.7
Second test period	TF	UK before	++				
		UK after	++	810	Pos (11×10 mm)	3.6	0.8
		UM before	++		See under		
		UM after	+++	6 200	Results	4.8	1.0
		KM before	+				
		KM after	+	2 600	Pos (10×10 mm)	5.3	1.3
Controls (9)							1.3 (mean value)

Candida albicans diluted 1/1000 (Vitrum, Stockholm, Sweden), 0,1 ml trichophyton vaccine (Sächsiches Serumwerk KG, Dresden, DDR) and 0.1 ml TF solution. The tests were read after 30 min and 72 hours.

Lymphocyte transformation tests were performed as described previously (7). The following mitogens and antigens were used: PHA 1 $\mu g/10^6$ cells/ml, ConA 50 $\mu g/10^6$ cells/ml, PPD 1 $\mu g/10^6$ cells/ml and herpes simplex antigen diluted 1/20. E-binding (T) rosette assays were performed as described previously (8) and the absolute numbers of T lymphocytes in peripheral blood were calculated after leukocyte and differential counting. Serum levels of IgE were determined by the PRIST method (commercial method, Pharmacia, Uppsala, Sweden).

RESULTS

The TF-batches used had a mean OD 260/OD 280 value of 2.46 (range 2.19–2.64) and the estimated peptide content varied between 16 and 38 mg/unit.

In the LTT, TF augmented the response to PWM and PPD in a dose-dependent manner with a decline at TF concentrations above and below the concentration giving maximum response.

The results of the therapy are given in Table I. No definite therapeutic effect of the TF treatment could be demonstrated in any of the patients. One of the patients (L. E.), who exhibited a marked tendency to secondary infections of the skin, demonstrated no such infections during the period of TF treatment. He experienced less pruritus but, although subjectively improved, the extent of the eczema was not reduced objectively. Another patient (U. M.) had an exacerbation of the eczema during the TF therapy and at the end of the therapy the skin tests were difficult to evaluate as the patient had developed erythrodermic skin.

In the immunological tests we found that the 4 patients who had previously been tuberculin skin

test negative had become positive after the TF treatment. In the other immunological in vivo and in vitro tests there were no significant changes.

Side effects

Patient K. H. complained of increased itching on the days following the 4th injection with TF and after the 5th injection she experienced urticaria and Quincke's oedema which abated within 24 h. The intracutaneous test with TF, which had been negative before the first TF injection, became positive (12×10 mm urtica surrounded by dendritic erythema). Control with saline solution was negative. There were no suspect provoking factors except for TF. The TF treatment was discontinued. Five weeks later the patient still had a positive i.c. test with TF. The 5 other patients were all skin test negative to TF, both before and after treatment. No other side effects were noted.

DISCUSSION

Successful use of TF therapy has been reported in two children with severe atopic dermatitis (1, 14). In one of the patients (14) the number of rosette-forming T lymphocytes was also reported to rise during the treatment. However, a later trial with TF treatment in this patient this time failed to alleviate the ezcema (6). Thulin et al. (18) gave TF (2–4 injections/month for 18 months) to 3 adults with atopic dermatitis and depressed cell-mediated immunity. They reported no major changes in the patients' atopic dermatitis and no prolonged effect was recorded in the immunological in vitro investigations.

In this investigation no clinical improvement could be demonstrated after TF treatment. Spontaneous exacerbations and remissions are common in atopic dermatitis and therefore a control group was also included. The 4 patients who were tuberculin negative changed to positive during the TF therapy. Repeated tuberculin testing in post-vaccinated patients may stimulate PPD reactivity (17) but as those in the placebo group remained negative, it is probable that this change to tuberculin positivity in the TF-treated patients indicates that the batches of TF used had some immunological effect in the patients.

In one patient the TF treatment was discontinued after the 5th injection because of a highly suspicious immediate hypersensitivity reaction against TF. To our knowledge, no such side effects have been reported previously. The risk of allergic reactions from TF has been said to be minimal, as TF consists of very low-molecular products (<10 000 Daltons) and is considered to be non-antigenic in man and animals (9). The TF batch used for this patient was subjected to gel filtration on Sephadex G-100 to determine whether high molecular weight aggregates had been formed during lyophilization. No material with an estimated molecular weight above 5 000 Daltons was found.

However, patients with atopy have a strong tendency to acquire reagin-mediated hypersensitivity and few patients with atopy have been treated with TF. Thus there may be a risk for hypersensitivity reactions against TF in this category of patients. In this investigation we were not able to confirm the promising results of TF therapy previously reported (1, 14). There may be many reasons to account for this.

It is unknown whether the depressed cell-mediated immunity in AD is primary or secondary. If the disturbances are secondary to the eczema, no clinical effects of TF therapy would be expected. Even if the disturbances in the immunity are primary, we still do not known where in the immune system the primary defect is located. Thus it is uncertain whether a drug, such as TF, can correct these immunological disturbances.

There are no good methods for testing the activity of TF for desired immunological effects. Thus different batches may have different activities and indeed both stimulative (3, 11) and suppressive (4, 12) activities have been found in chromatographic fractions of TF, using in vitro methods.

Another difficulty, due to the lack of methods to quantitate TF activity, is the schedule of treatment, i.e. the dose and the intervals between the injections. This may be very important, as many mechanisms in the immune system show a dose–response in regard to activating factors with a decline at concentrations above and below the concentration giving maximum response, e.g. high- and low-dose tolerance and mitogen responsiveness (10). This is also the case for the in vitro activity of TF in our experience. Therefore we believe that a true clinical evaluation of TF as a therapeutic agent can be accomplished only with a purified and clearly characterized substance.

ACKNOWLEDGEMENT

We are grateful to Mrs Monica Balodis and Mrs Eva Jansson for skilful technical assistance.

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Received February 24, 1978

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