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TRANSFER FACTOR THERAPY IN MYCOSIS FUNGOIDES: A DOUBLE-BLIND STUDY

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Abstract. Sixteen patients with mycosis fungoides (MF) were given either active transfer factor (TF) or heat-inactivated TF as additional therapy to topical nitrogen mustard or PUVA. The TF was prepared from non-selected healthy blood donors. The clinical evaluation after 2 years of therapy showed that among 8 patients treated with active TF, none went into complete remission of their disease, 4 patients had partial remission, one was unchanged, 2 progressed, and one died of active MF. In the placebo-treated group, 5 patients achieved complete remission and 2 partial remission. One patient died early in the trial due to cardiac disease. Immunological studies during the first year of therapy revealed cutaneous anergy towards tuberculin in most of the patients. This anergy did not change during TF therapy and differed from normal lymphocyte reactivity in vitro after tuberculin stimulation. At the start of treatment the patients had diminished levels of T lymphocytes in peripheral blood. A temporary increase was observed in the total number of T lymphocytes in patients after one month of treatment with active TF. After one year the T lymphopenia had disappeared in both groups. The mitogen reactivity of lymphocytes was found to be normal (PHA, PWM) or somewhat reduced (Con A). It is concluded that under the conditions employed in this trial, TF was not able to prevent progression of early mycosis fungoides, when viewed over a period of 2 years.

Mycosis fungoides (MF) is a rare cutaneous disorder which, after several years of confinement to the skin, often takes a progressive course with systemic involvement. The natural course is quite variable, but when extracutaneous spreading occurs, the disease is fatal (10, 22, 30). It is therefore of great importance to prevent or delay such a progression.

So far, treatment has been restrained by the unknown etiology of MF. Through histological, haematological and immunological studies, MF is now considered to be one of a group of diseases called cutaneous T cell lymphomas (CTCL) (7, 16). The abnormal cell is believed to be a T helper lymphocyte (2, 3, 31), but this still needs confirmation.

Earlier reports have indicated a reduction in cell-mediated immune reactivity in patients with MF (5, 6, 14, 17, 26, 28, 33). We have therefore used an immunopotentiating therapy (transfer factor, TF) as an additional treatment in patients with MF. The effect of such a therapy should hopefully prevent the proliferation of malignant cells and thus delay the progression of the disease.

In this report we present evidence that TF does not change the course of the disease, when viewed over a period of 2 years. In the patients studied there were signs of some immune deficiency, which was not reversed by TF therapy.

PATIENTS AND TREATMENT

Sixteen patients with MF participated in the study. A diagnosis of MF was only given when histological examination showed Pautrier's microabscesses in the epidermis. All biopsy specimens were reviewed by one pathologist (H. Søgaard). The stage classification was that of van Scott & Kalmanson (29).

The patients were randomized into two groups. Group I consisted of 5 women and 3 men, mean age 67. 4 years (range 53–83); Group II comprised 3 women and 5 men, mean age 65.0 years (range 47–82). Three persons in the first group and 2 in the second were included in the study because of a recurrence of MF after previous successful topical treatment. The rest of the patients were newly diagnosed cases of MF although many had experienced clinical symptoms for up to several years. Details regarding all the patients are given in Tables I and II.

Treatment

All patients received a standard treatment for MF, which is topical nitrogen mustard (40 mg per treatment (HN_2), initially given daily for 14 days, followed by treatment every week or second week until total clearance of the skin. Four patients were given PUVA because of severe hypersensitivity reactions towards HN_2 or to a relapse after previous HN_2 treatment. It was given two or three times weekly until remission. Maintenance treatment was given once weekly. One patient in stage III received electronbeam treatment only (patient U, Table I) and one patient in stage IV received topical nitrogen mustard and prednisone 5 mg daily (patient Ø, Table I).

Table I. *Mycosis fungoides patients treated with active transfer factor*
CR = complete remissio.., PR = partial remission, NC = no change, W = worse

Code	Sex	First clinical symptoms	Histological diagnosis	Age at diagnosis	Age at treatment	Treatment		Stage		
						Before TF	After TF	Before TF	After 1 year	After 2 years
E	F	1973	1975	50	53	PUVA, HN_2	HN_2	II	PR	NC
C	M	1972	1976	56	58	PUVA	PUVA	II	PR	PR
K	M	1976	1978	63	63	–	HN_2	II	PR	IV (cytostatic treatment)
I	F	1972	1978	72	72	–	HN_2	II	PR	PR
Z	F	1979	1979	77	77	–	HN_2	II	PR	PR
B	F	1969	1971	75	83	PUVA, HN_2	PUVA	II	PR	PR
U	M	1978	1979	74	74	–	Electron beam	III	Died after 5 TF inject.	–
Ø	F	1973	1978	59	59	HN_2, prednison 5 mg	HN_2, prednison 5 mg	IV	PR	IV (cytostatic treatment)
5 females 3 males				Range 53–83 years Mean 67.4 years					CR=0 PR=7 NC=0 W=1	0 4 1 2

TRANSFER FACTOR

Preparation

Buffy-coat cells were prepared from 450 ml of EDTA blood drawn from healthy, unselected blood donors. The donors were not skin tested, but approximately 90% of Danish blood donors have received Calmette vaccination during adolescence and between 1/2 and 3/4 are found to be tuberculin skintest-positive. The cells were transferred to 50 ml vials with 1 000 I. U. heparin. Each day buffy-coat cells from 8 or 10 donors were delivered. Each 50-ml vial was filled with 0.83% NH_4Cl, which lysed erythrocytes during 10 min incubation at 4°C. The leukocytes were washed twice in Hanks' balanced salt solution, pooled, counted, and stored at –20°C. On average, some 40–60% of the leukocytes were mononuclear cells, the rest being granulocytes. Approximately 1×10^9 leukocytes were prepared from 450 ml of blood.

The pool of leukocytes went through ten cycles of thawing and freezing (37° and –70°C) followed by ultrasonification for 60 sec. High-pressure filtration was performed using Amicon UM10 filters, which has a cut-off at 10 000

Table II. *Mycosis fungoides patients treated with heat-inactivated transfer factor*
CR = complete remisson, PR = partial remission, NC = no change, W = worse

Code	Sex	First clinical symptoms	Histological diagnosis	Age at diagnosis	Age at treatment	Treatment		Stage		
						Before TF	After TF	Before TF	After 1 year	After 2 years
L	F	1977	1978	47	47	–	PUVA	II	NC	PR
A	M	1977	1978	59	59	–	PUVA	II	CR	CR
P	M	1976	1979	62	62	–	HN_2	II	PR	PR
F	M	1979	1979	66	66	–	HN_2	II (d'emble)	CR	CR
H	F	1977	1978	67	67	–	PUVA	II	CR	CR
J	M	1968	1978	65	68	PUVA	HN_2	II	PR	CR
D	M	1973	1976	67	69	PUVA	HN_2	II	PR	CR
Æ	F	1974	1979	82	82	–	HN_2	II	Died after 4 TF inject.	–
3 females 5 males				Range 47–82 years Mean 65.0					CR=3 PR=3 NC=1 W=1	5 2 0 0

Table III. *Immunological investigations*

All patients were tested two or three times in vitro before TF treatment was begun. Blood was then drawn for investigations each second week for one month and then each third month

E-AET =Sheep erythrocytes, treated with 2-aminoethyl-isothio-uronium bromide hydrobromide (Sigma cat. no. A-5873), incubated with lymphocytes at 4°C for at least one hour. This technique gives the highest percentage for rosettes, approximately 80% (Table V; Control group). E-4 =Untreated sheep erythrocytes incubated with lymphocytes at 4°C for at least one hour. This technique gives approximately 60% rosettes (Table V; Control group). E-a =Untreated sheep erythrocytes incubated with lymphocytes for less than 5 min. This technique only reveals T lymphocytes with high affinity for sheep erythrocytes approx. 46% (Table V; Control group

In vivo

Tuberculin skin testing, 2 I.U per 0.1 ml performed before, 1–2 month after and one year after TF treatment was begun

In vitro

Total lymphocyte count per μl of blood

T lymphocytes	E-AET		
	E-4		
	E-active		
B lymphocytes, i.e. complement receptor bearing lymphocytes			
Mitogen reactivity in 3-day lymphocyte cultures:			
(*a*) phytohemagglutinin (PHA)	1.1	3.3	10.0 μg/ml
(*b*) concanavalin A (Con A)	8.0	25.0	μg/ml
(*c*) pokeweed mitogen (PWM)	1 : 600	1 : 200	of stock/ml
Antigen reactivity in 5-day lymphocyte cultures:			
(*a*) purified protein derivative of tuberculin	1.0	10.0	μg/ml

daltons. This gives a clear, water-like fluid, which after further filtration through a Gelman 0.22 μm disposable filter was divided into volumes containing 2 units of Transfer factor, 1 unit being equivalent to the extract volume from 1×10^9 mononuclear cells. The TF vials were stored at –20°C for up to 6–8 months until used. For simplicity we call our preparation TF, although it is dialysable leukocyte extract, which contains a vast range of moieties with diverse biological effects.

Administration of TF

We decided to give 1 unit of TF per week due to our previous knowledge about in vitro changes after TF (24, 32). For practical reasons 2 units were given each second week in conjunction with topical nitrogen mustard treatment. This treatment was given for one year. As placebo treatment, we used heat-inactivated TF (30 min at 56°C), which is not able to make a cutaneous transfer (15, 27). TF was given intramuscularly in the gluteal region. The side effects were slight or moderate pain at the site of injection, probably due to the hyperosmolarity of the solution, and a feeling of fatigue and unrest for 2–4 hours in one patient. In 3 patients we observed fever and malaise during a few hours, probably due to pyrogens. In 2 cases all subsequent batches of TF prepared on that particular day was destroyed, whereas the third batch of TF had already been given without any side effects. We never saw anaphylactic reactions.

Following one year of TF treatment the code was opened and all patients receiving active TF was given a maintenance dose of 2 units each third month. The patients receiving heat-inactivated TF did not receive any further placebo therapy.

Immunological investigations

Several immune parameters were followed on nine occasions before, during and after the TF therapy in order to check for any possible effect from TF and to correlate long-term immune observations with the course of the disease. The investigations performed are summarized in Table III. A detailed description of the immunological techniques is given elsewhere (13).

RESULTS

Among patients receiving active TF (Group I), none went into complete remission, 7 went into partial remission and one had died during the first year of treatment. Following 2 years of treatment, 4 patients continued in partial remission and one showed no change. Two of the patients in partial remission deteriorated and were therefore given systemic chemotherapy according to the Scandinavian Mycosis Fungoides Study Group (25) (Table I).

The patient who died (patient U, Table I) was a 73-year-old man, who presented with MF stage III with a clinical history of 5 months. He received a total of 5×2 I. U. of active TF and electronbeam therapy (total 35 Gy), before he suddenly developed uraemia with high serum levels of uric acid. An autopsy could not confirm the presence of uric acid crystals in the kidneys due to severe cadaverositas. His MF was considered to be active at the time of death.

Among patients receiving heat-inactivated TF (Group II) 3 went into complete remission, 3 into partial remission and one showed no change after one year's treatment. One patient (patient Æ, Table II) was an 82-year-old lady who died of cardiac disease just after TF was started. She had very low in vitro immune reactivity; however the results have been omitted. After 2 years of observation all

Table IV. *Tuberculin skin testing*

	Before TF	1 month after TF	1 year after TF
Active TF			
E	1.9	1.5	5.3
C	0	0.1	0.5
K	0	0	0
I	0.5	n.d.	0
Z	0	0	0
B	0	0	0.1
U	0	n.d.	Dead
Ø	0	0	0
Heat-inactivated TF			
L	2.1	1.9	1.9
A	0.2	0.2	0.1
P	0	0	2.5
F	n.d.	n.d.	n.d.
H	4.5	3.4	8.5
J	0	0	0
D	0.1	0.5	1.0
Æ	0	Dead	–

1 tuberculin unit was appplied to the dorsal side of a forearm and read after 48 hours. The area of induration was measured through planometry and given as cm^2.

patients were in partial or complete remission (Table II).

The results of tuberculin skin testing are given in Table IV. Only 2 persons in Group I were tuberculin positive before treatment, and only one became weakly positive after one year. Both the patient who died, and the 2 patients who deteriorated, were skin test negative. In Group II 4 of 7 patients had weak to strong tuberculin skin tests. The patient who died had a negative reaction.

The overall impression from the in vitro studies is the consistency found during 13 months. Before TF therapy the patients had a T lymphopenia (1 706 vs. 2 080 per μl; $p<0.05$, Student's t-test; Table V). The distribution of lymphocytes in blood showed that the percentage of T lymphocytes (E-AET and E-a rosette-forming cells) was within the normal range. Following the inception of TF therapy a temporary increase in the number of T lymphocytes was observed in Group I (Fig. 1). When comparing the values after 1 month of TF treatment with pretreatment values, no statistically significant difference was found. The T lymphopenia had disappeared after one year in both groups (Table V; Fig. 1).

The lymphocyte reactivity in vitro following PHA stimulation was normal before and after one year of TF therapy. The Con A reactivity was approximately 2/3 of the reactivity found in healthy persons and increased slightly after treatment. The PWM reactivity was slightly lower than in the control group. Most surprising was the positive and normal in vitro reactivity following PPD stimulation. When looking at the individual results, patients with a positive skin reaction had the highest in vitro reactivity and vice versa. In summary, in vitro reactivity of lymphocytes from the patients was normal or almost normal, when compared with lymphocyte reactivity in healthy persons. In the patients who deteriorated or died the in vitro reactivity was modest or low (12). In all investigations lymphocytes from patients in Group II had on average a slightly higher in vitro reactivity than lymphocytes from patients in Group I.

DISCUSSION

In a case report (32, 34) and in an open study (35) we have previously found that TF seemed to be of value as an additional therapy in early stages of MF. TF, which is a low molecular weight leukocyte extract, can transfer delayed skin reactivity to skin test negative healthy persons (15, 19, 21). It can also augment lymphocyte reactivity (8, 11, 18, 32).

We used TF because patients with MF have signs of decreased cell-mediated immunity. Delayed-type reactivity in the skin is reduced (28), a low percentage of T lymphocytes has been reported (17, 33), and a reduced lymphocyte reactivity in vitro has been observed in some studies, depending on the stage of the disease (5, 14, 17).

The present study is the first double-blind study of TF therapy in MF. The purpose of the treatment was to prevent or delay a progression of the disease from its cutaneous stages by increasing the immune reactivity in the patients. We found that TF was ineffective for that purpose, as only 4 persons went into partial remission, one was unchanged, 2 progressed and one died. This is in contrast to the placebo-treated group, where 5 patients achieved complete remission and 2 partial remission.

Our immunological investigations revealed a slight but significant T lymphopenia, which disappeared after one year's treatment. The almost normal immune reactivity in vitro is probably due to the early stage of the disease. When a severe progression took place, a reduced in vitro reactivity occurred (12). Most of the patients were tuberculin skin test negative and this did not change during TF

Table V.
The abbreviations in the left-hand column are explained in Table III. The mitogen restuls are given as counts per minute (cpm). All results are given as mean value ±1 S.D.

	Group I ("active TF")		Group II ("placebo")		
	Before (n=8)	After one year (n=7)	Before (n=7)	After one year (n=7)	Control group
E-AET per μl	1 783±666[a]	2 005±488	1 619±324[a]	1 967±707	2 080±690 (n=98)
E-a per μl	1 080±444	1 010±433	853±146	1 061±427	1 128±407 (n=95)
E-AET, %	78%±4.3%	77%±4.2%	77%±5.2	77%±3.7%	81 %±5.4 (n=121)
E-a, %	46%±10.2%	44%±5.3%	39%±5.6	42%±4.4%	46%±9.1% (n=121)
PHA	21300±14600	21300±14400	28000±13500	22500±6200	24300±10460 (n=123)
Con A	10200± 9100	12000± 8700	11900± 8500	15300±6200	18900± 9320 (n=105)
PWM	7400± 4200	8100± 4900	9600± 5800	8500±3400	10000± 5500 (n=105)
PPD	14800±10200	9400± 7300	15900± 9500	16700±9900	14600± 8470 (n=92)

[a] The mean value of T lymphocytes (E-AET per μl) in all patients (Groups I and II) was significantly lower than the mean value of T lymphocytes in the Control Group (p<0.05; Student's t-test).

treatment. However, all had PPD-reactive lymphocytes in peripheral blood, when tested in vitro. The cutaneous anergy may be due to the topical nitrogen mustard therapy, although one patient (E; Table I) had strong tuberculin skin reactivity even when she received that treatment. Two other patients with strong tuberculin reactions received PUVA. It is possible that topical nitrogen mustard, which was given before the first skin test, could inhibit weak to moderate tuberculin skin reactions, thus explaining the difference between our in vivo and in vitro findings. Our investigations also show that topical nitrogen mustard does not give systemic effects as regards the number of T lymphocytes in blood or the immune functions in the patients.

It may be argued that too little TF was given or that it was given for too short a time. In a recent study on TF therapy in multiple sclerosis, no effect of TF was seen until between 18 and 24 months, when a significant reduction occurred in the fre-

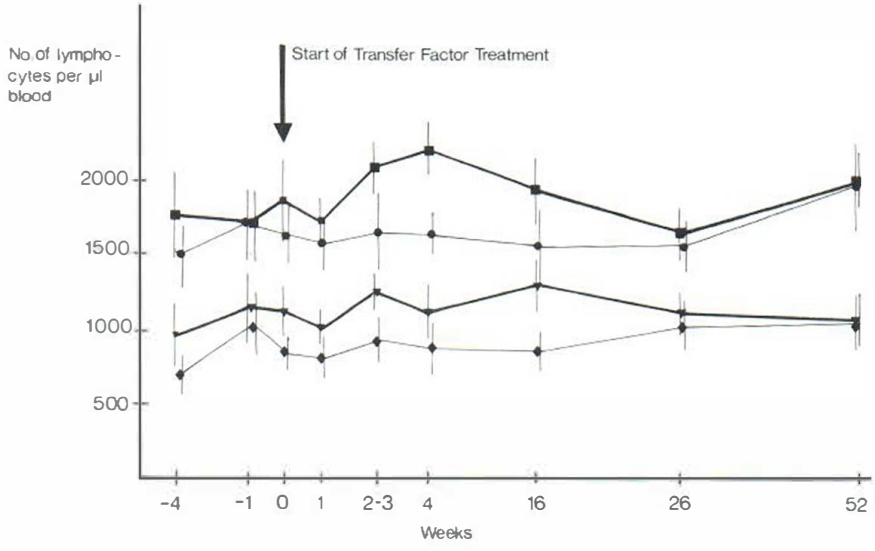

Fig. 1. The figure shows the total number of T lymphocytes in blood before and during TF therapy. ■, E-AET rosette forming lymphocytes, patients in Group I, treated with 'active' TF; ●, E-AET rosette forming lymphocytes, patients in Group II, treated with 'heat-inactivated' TF; ▼, E-a rosette forming lymphocytes, patients in Group I; ▲, E-a rosette forming lymphocytes, patients in Group II. The results are given as mean value ±1 S.E. of the mean (S.E.)

quency of disease recurrences (1). In a comparative study, where 21 of our TF-treated MF patients (stage II or III), were compared with 24 MF patients (stage II or III) from another Danish department, the survival rate was somewhat better for patients receiving TF and conventional therapy (3/21) than for patients receiving conventional treatment alone (8/24) (36). The patients were comparable as regards age. The TF-treated patients had received TF for 36 months or more.

Our preparation of TF is made in a similar way as described in the literature (4, 27). Many studies have shown that the dialysable leukocyte extract carries properties such as in vivo skin transfer (4, 15, 27), in vitro enhancement of E-rosette receptors on T lymphocytes (23), and in vitro augmentation of lymphocyte transformation (8). However, a preliminary study has stressed that various production procedures influence the amount of enhancing or inhibiting substances within the 'transfer factor' preparation (20).

We have not performed studies to describe the various in vivo and in vitro effects of our TF preparations, but are relying on available data from the literature. Because of our present results, one may speculate whether our TF preparations carry any activity at all. We can only say that our TF preparations are made in a similar way as described by other investigators. We looked at the skin conversion ability of TF, but were surprised to find a dichotomy between skin anergy, which could not be overcome by TF, and high in vitro reactivity of lymphocytes from blood towards the same antigen. We found, however, that the active TF preparation induced a slight increase in the total number of T lymphocytes within the first month of treatment (Fig. 1). Thirdly, our TF preparation is able to induce a pronounced increase in cAMP in T-gamma lymphocytes in vitro (9). At the moment we do not know if this finding is of any relevance to a possible in vivo effect of TF.

We used heat-inactivated TF because it is claimed to be incapable of transferring cutaneous delayed hypersensitivity (15, 27). It may still be capable of increasing in vitro functions of T lymphocytes or their number, as seen in Group II, where the T lymphopenia disappeared after one year's treatment (20).

One could make the criticism that non-specific TF was used and that heat-inactivated TF was used as placebo. However, the etiology of MF is not known and sufficient amount of TF from close contacts with the patients cannot be obtained.

Another question is how similar the two patient groups were. It is possible that patients in Group II had a better prognosis initially, reflected in their greater tuberculin skin reactivity and in their slightly greater in vitro reactivity (Table V). But we observed the relapses in those patients treated with 'active' TF, and this gives a more positive answer to our initial questions and reason for giving TF therapy.

Our previous experience with TF is that augmented immune reactivity takes place only when there has been a previous reduction. The apparent normal immune reactivity of the patients could then explain why no effect was seen from TF therapy. When MF progressed, diminished cell-mediated immune reactions occurred and at that stage of the disease a possible effect of TF should have been discovered.

Our conclusion is that a 2-year therapy with non-specific TF in patients with mycosis fungoides in the early stage cannot prevent further progression of the disease. Only long-term studies can resolve whether TF treatment can improve the survival of patients with MF.

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