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LONG-TERM TRANSFER FACTOR TREATMENT IN SEVERE ATOPIC DERMATITIS

By

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Since Lawrence demonstrated that delayed hypersensitivity reactions in man could be transferred by viable leucocytes or extracts hereof, called transfer factor (TF) (10), attempts to augment cell-mediated immune functions with TF have been made on patients with deficient cell-mediated immune reactivity. The main fields in which the clinical application of TF is used are infectious diseases (6, 9), immunodeficiency diseases (15) and malignancies (11, 20).

In dermatology TF has been successfully used to intensify parameters of cell-mediated immune reactions in a patient with severe skin infection caused by *Mycobacterium avium* (17); also in generalized vaccinia treatment with TF seems to be of value (5). In mycosis fungoides additional treatment with TF was found promising (21).

Within the last year two case reports have indicated a very successful use of TF in the treatment of severe atopic dermatitis in children (1, 16). We have treated three adults with severe atopic dermatitis and signs of depressed cell-mediated immune reactivity for a period of 1½ years. The present paper gives our results.

PATIENTS AND METHODS

Three adult patients, one male (P.D.) and two females (B.L. and B.M.) aged 31, 22 and 34 years respectively, with moderate to severe atopic dermatitis were selected for the trial. In the preceding 1½ years all three patients had been admitted to hospital; P. D. one, B.L. two, and B.M. three times for several weeks' duration, each time due to severe exacerbations of their disease including secondary infection of the skin. Their disease could only be controlled with potent topical steroids.

The patients had positive prick tests to several allergens, elevated IgE ranging from 2000 to 13000 units per ml and a tendency to repeated cutaneous infections. Tuberculin skin tests were weakly positive in all (1 T.U. induced from 3 to 8 mm induration). On one occasion, before initiation of TF treatment, the patients' lymphocytes were investigated for their mitogen responsiveness in a lymphocyte transformation test. Patients B.L. and B.M. showed normal reactivity to PPD (purified protein derivative of tuberculin) and various mitogens, whereas their response to a suboptimal concentration of concanavalin A (Con A) was significantly increased. Lymphocytes from patient P. D. gave diminished responses after stimulation with PPD, phytohaemagglutinin (PHA) and Con A, and he exhibited, at the time of investigation, a factor in serum which suppressed the reactivity of his own and allogenic lymphocytes. All had a subnormal number of T lymphocytes in the peripheral blood. A detailed report of the *in vitro* lymphocyte reactivity of these and other patients with atopic dermatitis has been published elsewhere (18).

Transfer Factor Therapy

One unit of TF corresponding to 1×10^8 leucocytes was given i.m. at intervals of 2 to 4 weeks. TF was prepared from pooled buffy coat cells from normal blood donors (21).

Clinical Evaluation

At each attendance TF was given and a clinical evaluation was performed based on parameters as to extent of skin involved (expressed in per cent of total body area), pruritus, lichenification, excoriations and erythema. Also, any signs of secondary infection (impetigo) were noted. The used amount and potency of topical steroid preparations were evaluated.

Immunological Investigations

Prior to TF injections blood samples were drawn for estimation of IgE concentrations, T and B lymphocytes, leucocyte migration test and differential and total leucocyte counts. Serum was secured for later investigation of immunosuppressive factors. These tests were performed regularly to measure the effect of TF treatment.

Ten healthy individuals had weekly estimations made of their T and B lymphocytes in blood during a 10-week period, including two periods of 5 days, when daily determinations of T and B cells in blood were made. Simultaneously, serum was prepared and the influence of autologous serum on leucocyte migration was studied on the day of investigation. The purpose of the investigations was to clarify the variations in these parameters in normal persons. All the healthy individuals were tested once for their response to PPD in the leucocyte migration test when AB serum was used as the serum source in the cultures.

Enumeration of T and B Lymphocytes

Samples of venous blood were processed for T and B cell counting as described in detail elsewhere (18). T lymphocytes were estimated as E-rosette-forming cells and B lymphocytes by counting EAC-rosettes (3). The absolute number of T and B lymphocytes per μ l blood was calculated from the differential counts of leucocytes in blood, the total number of leucocytes and the percentual T and B lymphocyte counts.

IgE Determination

This was carried out by solid-phase radioimmunoassay on serum samples (8) by Medicinsk Laboratorium, Copenhagen. The results are expressed in units per ml, 1 unit corresponding to 2 μ g/l.

Leucocyte Migration Test

The leucocyte migration test was performed using an agarose technique as described elsewhere (19). Leucocytes were suspended in Hepes-buffered tissue culture medium (TC-199) with:

- a) 10 per cent pooled AB serum and PPD.
- b) 10 per cent pooled AB serum and 0.9 per cent saline.
- c) 10 per cent autologous serum and PPD.
- d) 10 per cent autologous serum and 0.9 per cent saline.

PPD was supplied by Statens Serum Institut, Copenhagen, and used in a concentration of 250 μ g/ml.

After incubation at 37° C for 30 min, 7 μ l of the leucocyte suspensions were placed in wells on an agarose plate. Six cultures were set up with cells from each of the four suspensions and the agarose plates were incubated at 37° C in water-saturated atmospheric air. The areas of migration were measured by planimetry after 20 h incubation using a projection microscope. The following ratios or migration indices (MI) were calculated:

MI-1: Mean migration area in cultures with AB serum and PPD/mean migration area in cultures with AB serum and saline.

- MI-2: Mean migration area in cultures with autologous serum and PPD/mean migration area in cultures with autologous serum and saline.
- MI-3: Mean migration area in cultures with autologous serum and saline/mean migration area in cultures with AB serum and saline.

Immunosuppressive Factors in Serum

Investigations for immunosuppressive factors (ISF), which would suppress a proliferative response of normal lymphocytes, were performed on the same day using serum samples drawn from the patients at various dates during TF therapy. Lymphocytes from two normal donors were stimulated *in vitro* with three increasing concentrations of PHA, the highest concentration was known to induce a maximal proliferation of lymphocytes in the culture system used. The assay for ISF has been described elsewhere (18). Serum samples, drawn on different dates from normal donors, were included to see if the storage time of serum could influence the results.

RESULTS

Clinical Evaluation

An evaluation of the clinical status throughout the observation period showed no changes in patient B.M., slight improvement in patient P.D. and significant improvement in patient B.L. as compared with their status before treatment. It should be noted, however, that none of the patients were admitted to the hospital during the period of treatment. Also, a general shift was seen from prior use of a highly potent topical steroid preparation to a medium strength preparation. During the first 6 months of TF therapy all patients had one or two attacks of severe impetigo lasting for approximately a week, but none of them suffered severe attacks of cutaneous infections during the last year of treatment.

IgE Determinations

The IgE values in all the patients were significantly higher than the normal range ($P < 0.001$). The mean values and the coefficients of variation ($CV = \text{s.d./mean}$) for each patient, based on a number of determinations during the period of treatment, appear from Table 1. Characteristically, each patient maintained a rather constant level of IgE in serum during

TABLE 1
Mean Values and Coefficients of Variation (in Parentheses) for Each Patient and Control Calculated from a Number of Consecutive Determinations during TF Treatment

	Patients				Controls			
	P.D.	$\bar{x}$	CV	B.M.	$\bar{x}$	CV	B.L.	CV
IgE (units/ml)	3994*	3994*	(18.0)	2362*	12933*	(29.0)†	12933*	(18.0)
T lymphocytes/ μ l	511*	511*	(36.0)	890*	967*	(33.0)	967*	(30.0)
B lymphocytes/ μ l	391*	391*	(32.0)	431*	590†	(32.0)	590†	(39.0)
PPD-responsiveness	0.94	0.94	(11.9)	0.94	0.98	(10.1)	0.98	(9.7)
Inhibition by autol. serum	0.97	0.97	(9.3)	1.01	1.00	(10.7)	1.00	(10.6)

Student's *t*-test.

*: $P < 0.001$.

†: $P < 0.05$.

$\bar{x}$: mean value.

CV: coefficient of variation (s.d./mean).

() Based on the results obtained from the same individual.

[] Based on the results obtained from different individuals.

The concentrations of IgE are given in units per ml, each unit corresponding to 2 μ g/l. T and B lymphocytes are given as absolute numbers of peripheral cells per μ l. The activity of a serum inhibitory factor is expressed as the ratio between the leucocyte migration areas in autologous serum and in AB serum. The leucocyte responsiveness to PPD is calculated as the ratio between the migration areas in AB serum with PPD added and in AB serum alone.

TF therapy, although some fluctuation was seen. This fluctuation only differed significantly ($P < 0.05$) in one patient (B.M.) from the expected variation at that IgE level. To calculate the expected variation we used the CV from IgE determinations in a group of normal persons. With this percentage, we obtained an expected standard deviation (s.d.e.) and established the range of "normal" variation for each patient (mean value of IgE $\pm 2 \times$ s.d.e.).

T and B Lymphocytes

TF treatment did not change the number of T and B lymphocytes in blood significantly. The absolute number of T cells remained subnormal in patients B.M. and B.L. throughout the period and was consistently low in patient P.D. ($P < 0.001$). A similar decrease was seen in the absolute number of B lymphocytes ($P < 0.005$ and $P < 0.001$) (Table 1).

The variation in the absolute number of T cells in each patient during the TF treatment was rather pronounced, but not significantly different from the variation in the control group. It should be noted that the mean value and CV for the T- and B-cell determinations in the control group are based on week-to-week determinations. The CV value for the day-to-day determinations of T and B cells in this group (8 per cent for T lymphocytes and 4 per cent for B lymphocytes) was statistically significantly different from the CV value of the week-to-week determinations.

Leucocyte Migration Test

Fig. 1 shows the individual results from each patient of their leucocyte migration *in vitro* following exposure to PPD. The range in the Figure is the mean migration index $\pm 2 \times$ s.d. from the control group of healthy individuals, all of whom were Mantoux positive. Most of the migration indices lie in the upper part of the normal range, indicating only slight inhibition. However, it should be noted that low values of MI were recorded early on during the TF treatment which could

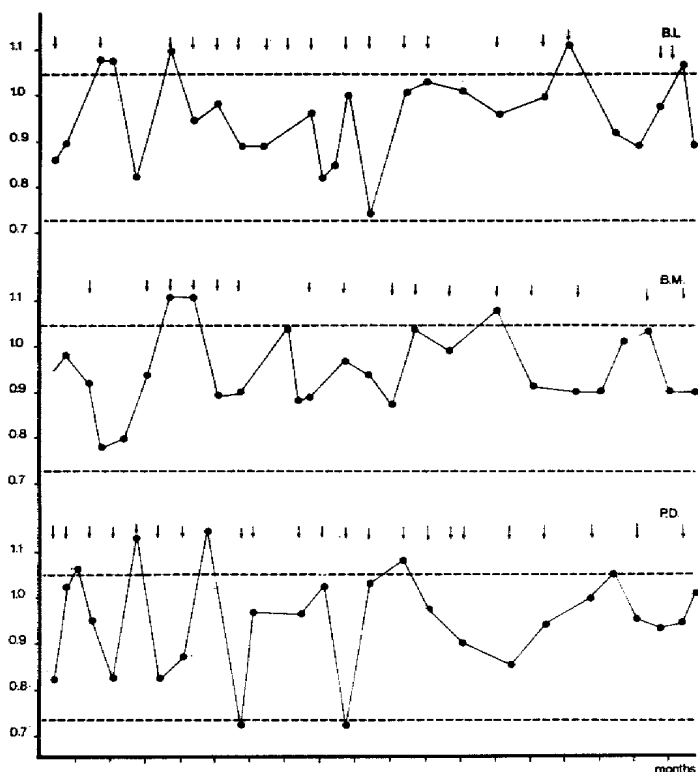


Fig. 1.

Variations in cellular immunity to PPD as determined *in vitro* by the leucocyte migration test. The results are expressed as ratios between the migration areas in cultures with AB serum and PPD and with AB serum alone. The range for normal persons is indicated as the average migration index $(0.88) \pm 2 \times \text{s.d.}$ (0.88). Arrows indicate the times of transfer factor injections.

be due to the effect of TF. The mean values of MI and CV are listed for patients and controls in Table 1.

The mean migration area in cultures with autologous serum and PPD was not different from the mean migration area in

cultures with AB serum and PPD, both in patients and controls.

To find serum factors which could inhibit the migration of leucocytes we evaluated the ratio between the migration area of leucocytes in cultures with autologous serum and saline and the migration area in cultures with AB serum and saline. This index should be 1.00, if no inhibitory effect is induced through autologous serum. No statistically significant differences were found between the mean values of the indices from patients and controls (Table 1), but an inhibitory effect could be found in some individual serum samples during early TF treatment. This inhibitory activity occurred concomitantly with severe skin infections. P.D. showed indices of 0.79, 0.73 and 0.85; B.M. had indices of 0.73, 0.87 and 0.88; and B.L. had 0.85, 0.86 and 0.81 compared with their mean values of 0.97, 1.01 and 1.00 respectively.

Immunosuppressive Factors

The addition of patients' serum to lymphocyte cultures from two normal donors did not reveal any definite signs of ISF. With the lowest concentration of PHA the proliferative response was clearly diminished in cultures with serum obtained from patient P.D. during his first 6 months of TF therapy. But this pattern of depressed reactivity was not found when higher concentrations of PHA were used. B.M. and B.L. showed no signs of ISF, although B.L. had had some inhibitory activity in her serum samples in February 1976. This was not clearly related to exacerbation of skin lesions.

DISCUSSION

The purpose of the present trial was to evaluate the influence of TF therapy by clinical and immunological parameters in three selected patients with severe atopic dermatitis and signs of depressed cellular immunity. Following 1½ years of TF therapy we found the following: IgE concentrations in serum remained high with no trend toward decrease. The absolute

number of peripheral T and B lymphocytes remained subnormal and no influence of TF upon the number of T and B cells could be demonstrated. *In vitro* reactivity to PPD seemed to be intensified to some degree during the early period of treatment, but the effect gradually faded out. Serum factors which inhibited leucocyte migration could be demonstrated simultaneously with severe attacks of cutaneous infection and disappeared at the same time as the tendency to impetigo ceased. On the other hand, there was no clear evidence for serum factors which could inhibit the proliferation of normal lymphocytes in a lymphocyte transformation test. Some clinical improvement was noted, the most important being the absence of impetigo; admission to hospital was also avoided.

High concentrations of IgE in serum are consistently found in patients with atopic dermatitis and a correlation between the IgE level and the severity of atopic dermatitis has been proposed (4, 7, 13). In the present study, however, the fluctuations in IgE levels did not seem to correlate closely with the clinical status of the patients.

Low numbers of T lymphocytes in severe atopic dermatitis has been demonstrated by many authors (2, 12, 18). An induced increase in the procentual number of T lymphocytes accompanied by clinical improvement has been reported in a child following TF treatment (16). This was not seen in our patients.

An interesting finding was the marked fluctuation in the absolute numbers of T and B lymphocytes based on week-to-week results in patients and control persons. This fluctuation is not likely to be due to technical variation, since the day-to-day fluctuation expressed as CV was found significantly smaller than the CV for the week-to-week determinations. The reason for this fluctuation is unexplained, but it should be kept in mind when determinations of T lymphocytes have only been done for shorter periods of 1 or 2 months and only few results are available.

The migration inhibition of the patients' leucocytes after exposure to PPD was rather weak. Following the start of TF

treatment, increased inhibition of leucocyte migration was sometimes observed in the cultures containing PPD, but this phenomenon gradually ceased. We have observed a similar pattern of changes in a lymphocyte transformation test during long-term TF treatment of a patient with *Mycobacterium avium* infection (Thestrup-Pedersen & Thulin, unpublished results).

The inhibition of leucocyte migration by autologous serum during the early period of treatment occurring simultaneously with attacks of impetigo, could probably be related to a factor which inhibits certain properties of phagocytic cells. Thus, Rogge & Hanifin (14) have described the presence of serum factor(s) in atopic patients which depress chemotaxis of phagocytic cells and inhibits transformation of lymphocyte *in vitro*. However, the serum factor(s), which in the present study were found to inhibit leucocyte migration, was not correlated with inhibition of lymphocyte proliferation. Also, we could not demonstrate any correlation between the presence of "migration-inhibition-factor" in serum and the increased inhibition of leucocyte migration occasionally seen after addition of PPD to the cultures.

TF, prepared in the same way as in this trial, has earlier been used successfully in our department for the treatment of mycosis fungoides (21) and in a patient with generalized *M. avium* infection (17). However, in the present study no lasting effect with TF treatment was recorded in the *in vitro* investigations and only minor clinical improvements were obtained. We are therefore not able to confirm the promising results of TF therapy in children with atopic dermatitis (1, 16), but one important difference may be that our patients were adults whereas the two successfully treated patients were 9- and 12-year-old girls.

SUMMARY

Transfer factor therapy was applied in three patients with severe atopic dermatitis and given at regular intervals for 1 1/2

years. Clinically, slight improvements were seen, attacks of impetigo ceased and admissions to hospital were not necessary. However, IgE concentrations in serum remained constantly high in all cases and the absolute number of T and B lymphocytes was continuously subnormal despite treatment. The *in vitro* cellular reactivity to PPD as assayed by a leucocyte migration test was not significantly altered in the patients, although a slight increase was found early on in the therapy. Finally, a serum factor inhibiting leucocyte migration and appearing simultaneously with attacks of impetigo disappeared during treatment.

In conclusion, no convincing effect of transfer factor therapy was encountered in immune parameters and no major alterations were found in the status of the patients' atopic dermatitis.

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