Eur J Cancer Clin Oncol, Vol. 24, No. 5, pp. 929–933, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00

Transfer Factor in Hodgkin's Disease: a Randomized Clinical and Immunological Study

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Abstract—*Transfer factor (TF) was prepared from buffy coats obtained from 493 units of blood taken from healthy donors, including individuals convalescent from various viral infections. It was administered to 22 of 47 patients with Hodgkin's disease undergoing treatment and consenting to take part in this randomized study to determine if TF would enhance their immunity and/or reduce the incidence of subsequent infections. Skin test reactivity was markedly enhanced in those patients receiving TF as opposed to placebo but other immunological assessments showed no significant differences between the groups. TF was not shown to be of benefit in the prevention of infections (including varicella/zoster).*

INTRODUCTION

PATIENTS with Hodgkin's disease are known to have defects in immunity which may be further depressed by radiotherapy and/or chemotherapy leading to an increased susceptibility to infection [1]. Transfer factor (TF) is a low molecular weight dialysable lymphocyte extract present in the lymphocytes of immune subjects which may be extracted and inoculated to produce a corresponding immunity in anergic recipients. The use of TF in the transfer of delayed cutaneous hypersensitivity to tuberculin to non-immune recipients was first reported by Lawrence [2]. In uncontrolled trials TF therapy has improved cell mediated immunity in Hodgkin's disease [3, 4] and has also in a controlled study reduced the incidence of varicella/zoster infection in childhood leukaemia [5]. We have carried out a controlled randomized study of TF in patients with Hodgkin's disease undergoing treatment to determine if it will enhance their immune status and reduce the incidence of infection.

METHODS

Transfer factor (TF) was prepared from the buffy coat of whole blood supplied by Sheffield Regional Blood Transfusion Service who used a differential centrifugation technique. The buffy coat was freezed/thawed five times to disrupt the cells and aseptically dialysed (8000 MW filter) against × 20 volume of distilled water. The dialysate was concentrated by freeze-drying and the product reconstituted with sterile distilled water at a concentration such that 1 ml was obtained from 2×10^8 mononuclear cells (the international standard for TF is $1–3 \times 10^8$ cells per ml). It was stored frozen, after being checked as non-pyrogenic. The placebo was Haemaccel (Hoechst), a plasma expander. TF was prepared from buffy coats extracted from 493 units of blood taken from healthy donors including individuals convalescent from various viral infection (25 herpes zoster, 32 varicella, 13 rubella, 5 measles, 1 mumps, 1 herpes simplex).

A total of 47 patients consented to take part in the randomized study; 22 were allocated to receive TF, 25 placebo. Four patients were withdrawn from the study on the grounds that review of their histology showed non-Hodgkin's lymphoma rather than Hodgkin's disease (1 in the placebo and 3 in the TF group). According to their randomization, patients were inoculated subcutaneously with 1 ml of TF or placebo; this was done on a double blind basis. The inoculations were repeated at 3 month intervals over a period of 21 months during which time the patients were followed up regularly at the lymphoma clinic. The dosage of transfer factor and 3 month injection period was chosen on the basis of other previous investigators' experience. The clinical features of patients in the study are summarized in Table 1. Delayed hypersensitivity (tested by intra-dermal injection of recall antigens) was

Accepted 5 January 1988.
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Table 1

Patients	Transfer factor 19	Placebo 24
Stage		
1	4	2
2	9	10
3	4	10
4	2	2
Symptoms		
A	13	16
B	6	17
Histology grade		
I	12	12
II	7	12
Skin test* positivity at presentation		
Candida	3	3
Mumps	5	6
Tuberculin	7	9
Streptokinase	4	6
Splenectomy		
+	3	4
−	16	20
Radiotherapy	6	8
Chemotherapy	8	11
Both	5	5
Complete remission	14	20
Partial remission	3	3
Non response	2	1

Staging by Ann Arbor criteria [6].
Histology by British National Lymphoma Investigation criteria [7].
*Skin test data available on 16 TF and 18 placebo treated patients.

assessed before initial inoculation and at the end of the study. *In vitro* tests of immunity were performed on blood samples collected before inoculation of TF or placebo, 48–72 h after inoculation (the traditional time to assess 'recall' cellular immunity) and before inoculation at 3, 9, 15 and 21 months.

Tests of Immunity

Cellular immunity

Skin tests. Intra-dermal skin tests were performed with the following recall antigens: *Candida albicans*, mumps, tuberculin and streptokinase/streptodornase. The test sites were evaluated at 48 h and the diameter of any induration measured; 5 mm or more was considered positive.

Leucocyte migration inhibition (LMI) test. The LMI test was modified from the method of Søberg and Bendixen [8]. Separated peripheral leucocytes were allowed to migrate from micro-capillary tubes in wells containing (a) purified protein derivative (PPD), (b) *Candida albicans* extract and (c) control medium. A migration index was determined by dividing the area of migration with antigen by the area of migration without antigen. A patient was said to be positive for this test when the migration index was 0.80 or less.

Lymphocyte transformation. The technique used was modified from that of Schellekens and Eijsvoogel [9]. Mononuclear cells were separated from whole blood by a Ficoll-based centrifugation method; washed three times in RPMI1640 tissue culture medium (Flow) and distributed into triplicate cultures containing culture medium, autologous plasma and phytohaemagglutinin (PHA) diluted 1:25. Control healthy lymphocyte cultures were also set up, and all cultures were incubated at 37°C for 72 h. Lymphocyte transformation was assessed by measuring the uptake of tritiated thymidine during the last hour of incubation. DNA synthesis was then arrested by cooling to 4°C and the cells were harvested using a Skatron cell harvester. Radio-activity was counted to give a final result in disintegrations per minute (dpm).

T-cell counts. The spontaneous E-rosette formation technique used was based on that of Steel *et al.* [10]. Washed, fresh, defibrinated, preservative-free sheep red cells were added to washed, separated mononuclear cells in culture medium, centrifuged at 200 ***g*** for 5 min and incubated at 4°C for an hour. After resuspension the proportion of rosette forming lymphocytes was counted in a haemocytometer.

Humoral immunity

B-cell counts. Separated mononuclear cells were washed three times in RPMI 1640. One drop of a 1:10 dilution of fluorescent anti-sera (fluorescein conjugated goat anti-human immunoglobulin, Tago) was added to 100 μl aliquots of 2.5×10^5 cell suspensions. The tubes were then rotated at 4°C for 30 min. The proportion of fluorescing lymphocytes was then counted in a wet preparation using a Leitz Dialux microscope fitted with a Pleompak 23 illuminator.

Immunoglobulins. Serum immunoglobulins were kindly determined using automated immuno-precipitation by the Department of Immunology, Royal Hallamshire Hospital as part of the established monitoring of these patients.

Peripheral blood counts

Full and differential white cell counts were performed at each stage of the assessment.

Table 2. Incidence of infection in 2-year follow-up

	Transfer factor (19)	Placebo (24)
Bacterial		
Septicaemia	2 (1*)	—
Other	2 (1*)	3 (2*)
Viral		
Zoster/varicella	3	4
Minor	5	8
Fungal		
Candida	—	1

*Patient died with disseminated Hodgkin's disease.

Table 3. Change in skin test status at completion of study (total 28 patients)

	Response	Transfer factor (14)	Placebo (14)
Response to individual antigens	↑	5	2
	↓	1	1
Number of positive skin tests	↑	3	1
	↓	0	1
Both of above	↑	4	1
	↓	0	2
Total	↑	12	4
	⟷	1	6
	↓	1	4

Key: ↑ increased, ⟷ unchanged, ↓ decreased } compared with pre-inoculation assessment.

Monocytes IgG Fc receptor (γFcR) assay. The proportion of blood monocytes bearing γFcR was assayed on the purified mononuclear cell preparations. Monocyte γFcR rosettes were formed using ox erythrocytes coated with anti-ox IgG [11], cytofuge preparations were made, stained for non-specific esterase and the percentage of monocytes expressing γFcR determined.

Infections

Patients were asked to keep a careful diary of any infections developed during the period of follow-up and all these were documented in the patients' records. The form of treatment and the clinical response (complete, partial or non-remission) were recorded.

Statistical analysis

The results in TF and placebo groups were recorded for each assessment of immunity. As a first step absolute values were compared within and between groups using appropriate Student's t and paired t tests. Patients were then assessed as to the relative changes in levels for each of the variables for each main factor (i.e. TF or placebo) using a two-way analysis of variance [12]. Values were considered significant if $P < 0.01$. For comparison of data on skin tests and incidence of infection chi^2 (with Yates correction) values were calculated.

RESULTS

Table 1 shows that patients in the two arms of the study were well balanced in terms of the clinical stage, histology grade, splenectomy status, treatment received and response to treatment. There was no significant difference ($chi^2 = 0.32$; $P > 0.5$; 1 df) in the incidence of infections between the two arms of the study (see Table 2). Two patients in each arm of the study refused further inoculations after 6 months but consented to having further blood samples taken off for immunological testing. These results are included in the analysis. One patient in the TF group did not complete the study due to fatal relapse of his Hodgkin's disease.

1. *Skin tests*

Since some patients were unwilling, either at presentation or follow-up, to have skin tests (though otherwise consenting to participate in the study) complete data on skin test assessment was available on only 28 patients (14 TF, 14 placebo) (Table 3). In those patients receiving TF enhanced responses (judged either by an increase in the positivity to specific antigens and/or to an increased number of positive tests) was seen in 12 patients, one showed deterioration and one was unchanged. In those patients that received placebo, 4 patients showed enhanced responses, 6 remained unchanged and 4 showed deterioration. These differences between the groups were significantly different ($chi^2 = 7.15$; $P<0.01$; 1 df). In 3 patients who had received TF, post treatment skin test reactivity was clinically judged supranormal (i.e. prolonged marked induration, redness and tenderness); no such responses were seen in the placebo group. Interestingly, before treatment 5 patients in the TF and 5 in the placebo group were totally anergic to skin testing. At the end of the study only one patient in the TF (compared with 3 in the placebo) group was still totally anergic. Discordant skin test reactivity (i.e. increased reaction to one antigen and decreased to another in the same individual) was not observed.

2. *Leucocyte migration inhibition index*

No significant changes in indices were observed either within or between groups. Twenty-nine per cent of the patients tested at presentation reacted to either PPD or *Candida*, consistent with previous findings [13] where 31% of patients reacted compared with 55% of normal controls. Only 6 patients

in each arm of the study did not show any positive response to either antigen at any time. During sequential assessments in the TF group 5 patients gave a positive response with PPD and 7 with *Candida* after being negative to these antigens at presentation. In the placebo group 7 patients changed from negative to positive with PPD and 7 with *Candida* at some time during the study.

3. *Lymphocyte transformation*

Significant falls in values for lymphocyte transformation occurred in both TF and placebo treated groups and these persisted throughout the study. No significant differences between groups were observed.

4. *Lymphocyte sub-populations*

The proportion and absolute number of T cells fell with treatment and remained depressed throughout. No significant differences were noted between the TF and placebo treated groups. No significant changes in B cell numbers or proportions were seen in either group or between groups.

5. *Immunoglobulins*

IgG. Falls from presentation values were seen in both TF and placebo treated groups following treatment. The difference was significant only between presentation and three months values in the TF treated group.

IgA. Falls were seen in values, being significant after treatment in the placebo treated group.

IgM. Falls were seen in both groups but the mean value was significantly different only at 3 months in the TF treatment group.

There were no significant differences between groups at any stage for any of the immunoglobulin classes.

6. *Blood count*

Lymphocyte counts fell following treatment with subsequent recovery; these falls did not reach statistical significance however. No differences were observed between the TF and placebo groups. Neutrophil counts showed the expected fall following treatment (reaching significance in the TF treated group) and these were persistent throughout the study. Monocyte counts showed no significant changes.

7. *Monocyte IgG Fc receptor assay*

No significant changes or differences were observed.

The laboratory data were also assessed as relative changes at each stage (see statistical analysis). The findings were essentially the same as for absolute value analysis, i.e. significant ($P < 0.01$) falls in T cell numbers (both groups), neutrophil counts (TF group), lymphocyte transformation (TF group) and immunoglobulin values (IgG, A and M in the TF and A in the placebo group) and no significant change with leucocyte migration inhibition or lymphocyte and monocyte counts. As with absolute value analysis there was no significant difference between the TF and the placebo treated groups for any of the tests at any follow-up assessment except for the relatively better leucocyte migration inhibition response to PPD in the TF treated group at final assessment.

DISCUSSION

Passive transfer of delayed hypersensitivity with TF has been observed in patients with Hodgkin's disease in remission; the cellular immune responses of the recipients improved in terms of skin test reactivity to both recall antigens and to dinitrochlorobenzene, suggesting both specific and non-specific immune enhancement with the TF [3].

TF has been used to treat varicella zoster infection in lymphoproliferative malignancy both by itself and together with zoster immunoglobulin [14]. In Winsnes *et al.*'s pilot study [14] therapy with the TF alone and particularly in combination with the zoster immunoglobulin seemed to have a beneficial effect when administered early; an increase in serum interferon in some of the patients receiving TF was also found. Steele *et al.* [5] in a double-blind trial designed to examine the clinical efficacy of TF showed that the active preparation was capable of preventing varicella zoster infections in patients with childhood leukaemia. TF converted negative skin test results for varicella zoster to positive in approximately half of the recipients.

These studies point to a multiplicity of immunological actions for TF and there is lingering controversy over the specificity and mechanisms of effect [15] and our study does not help resolve this. We found no clinical benefit, in terms of reduced incidence of infection, in patients receiving TF. In the tests of immune function, the expected depression of immunity following treatment with radio and/or chemotherapy [1] was seen.

However no differences in immunological assessments were seen between TF and placebo treated patients assessed up to 21 months following treatment with the exception of marked enhancement of skin test reactivity in those patients treated with TF. These findings support those of Heinonen *et al.* [16], who found that TF intensified skin test reactions in patients in remission of their lymphoma, but had no effect on the other tests of cellular and humoral immunity.

This small trial provides further evidence for the immunostimulatory nature of TF on certain aspects

of cell mediated immunity. However TF apparently did not have any effect in preventing infections (including varicella/zoster) in patients with Hodgkin's disease undergoing treatment.

Acknowledgements—The help of the following people is gratefully acknowledged: Mr. A. Moore, Miss J. Martin (Pharmacy), Dr. R. van Noort, Mr. G.A. Harston, Miss C.A. Segasby (Medical Physics and Clinical Engineering), Mr. M. Gascoign, Mr. D. Ashton, Mr. D.N. Arkley, Mr. R.R. Stapleton (Regional Blood Transfusion Service), Dr. L.H. Ferguson (Medical Microbiology), Mrs. L. Cawood, Mr. M.D. Whitham (Medicine) and Sister M. Breeze, Dr. I.H. Manifold and Dr. M. Aitken (Weston Park Hospital). The project was financially supported by the Yorkshire Cancer Research Campaign.

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