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No effect of transfer factor in juvenile rheumatoid arthritis by double-blind trial

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SUMMARY A previous pilot study of treatment with transfer factor in 3 patients with juvenile rheumatoid arthritis (JRA) gave promising results. However, in this small and open study no definite conclusions could be drawn. Therefore, a double-blind group trial was performed in 12 JRA patients treated with transfer factor, and in 12 placebo-treated control patients. The patients were evaluated clinically, by laboratory tests, and by estimation of different lymphocyte populations and cell-mediated immunity *in vitro* and *in vivo*. Transfer factor was not found to be of significant therapeutic value in patients with JRA. The only statistically significant difference between the two groups was a greater reduction in the percentage of T lymphocytes in transfer factor-treated patients than in control patients. The significance of this is difficult to explain and could have appeared by chance. No side effects of treatment with transfer factor were noted.

In a previous open study 3 patients with severe juvenile rheumatoid arthritis (JRA) were treated with 5 to 10 injections of transfer factor for 3 to 5 months (Frøland *et al.*, 1974; Kåss *et al.*, 1974; Natvig *et al.*, 1976). No flare-up of the disease or clinical progression occurred, but there was general clinical improvement and in 2 cases joint function improved as well. There were some concomitant changes in cell-mediated immunity, with conversion of previously negative responses to antigens in delayed hypersensitivity *in vivo* and lymphocyte transformation *in vitro*.

These results may not have been due to the effect of transfer factor, however, but could have been caused by factors such as spontaneous recovery, other treatment, placebo effect, milieu factors, or bias or error in evaluation of the different parameters. We therefore decided to carry out a controlled clinical trial. Because of the relatively large fluctuations in disease activity with time, a parallel rather than a cross-over trial was conducted, with one group treated with transfer factor and one group treated with placebo (Hill, 1969; Amor *et al.*, 1974).

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We present the results of the clinical and immunological evaluation of 12 patients treated with transfer factor and 12 patients treated with placebo. Immunological evaluation included *in vitro* stimulation of lymphocyte reactivity and delayed hypersensitivity skin testing as well as peripheral blood lymphocyte population studies.

Patients

The 24 patients were diagnosed according to the criteria of Ansell and Bywaters (1969). Patients in complete remission and those with very severe disease possibly requiring surgical treatment or a change in medical treatment during the trial were excluded. A minimum of 4 weeks elapsed between an operation and the start of the trial. None of the patients had had skin tests with any antigen preparation during the preceding 3 months. The mean age of the patients was 11·6 years (range 7 to 16 years). 3 patients belonged to functional class I and 21 to class II according to Steinbrocker *et al.* (1949). The type of onset, according to the criteria of Calabro and Marchesano (1968), was polyarticular in 19, mono/oligoarticular in 4, and acute febrile type of the disease in 1. Serum rheumatoid factor was found in 2 cases by the Rose–Waaler test. 4 patients had a positive antinuclear factor test using

mouse liver as substrate. 2 patients had amyloidosis verified by Congo red staining and immunohistochemical staining of liver biopsy preparations (Husby *et al.*, 1973).

Treatment was unaltered during the trial. Before and during the trial 22 patients received salicylates, 1 naproxen, 4 chloroquine preparations, 1 a maintenance dose of gold salts, and 3 low-dose corticosteroids. The trial was carried out on outpatients as well as inpatients at the Oslo Sanitetsforening Rheumatism Hospital between June 1974 and August 1975. Informed consent was obtained from the patients and their parents.

Materials and methods

TRANSFER FACTOR AND PLACEBO

Transfer factor was prepared from 250 to 600 $\times 10^6$ lymphocytes obtained from 400 to 450 ml venous blood (heparin 0·01 IU/l) from normal adult blood donors. Details of cell extraction, freezing and thawing, dialysis, and lyophilisation were as described previously (Frøland *et al.*, 1974). The dialysates containing transfer factor were then dissolved in 4–5 ml distilled water and sterilised by passage through a 0·22 μm millipore filter. Physiological saline was used as placebo. The injections were made subcutaneously in the deltoid area.

DESIGN OF TRIAL

The trial was double blind. The patients were randomised so that 12 received injections of transfer factor and 12 placebo. Only the statisticians and a nonmedical person who stored the preparations in a −20°C freezer knew the code until all the clinical and laboratory tests had been evaluated. The observation period was 6 months. The schedule of treatment and evaluation is given in Table 1. Each patient received injections every fortnight for the first 3 months and thereafter at monthly intervals. Clinical evaluations and several laboratory tests were performed monthly. Estimation of lymphocyte populations in peripheral blood and evaluation of cell-mediated immunity *in vitro* were performed before the study and after 3 and 6 months, whereas testing for skin hypersensitivity was only performed before and after completion of the study.

Table 1 *Treatment and evaluation schedule for a 6-month parallel group trial of transfer factor in juvenile rheumatoid arthritis*

	Start	*Month after start of treatment*					
	0	*1*	*2*	*3*	*4*	*5*	*6*
Transfer factor or placebo	×	×	×	×	×	×	×
Clinical and laboratory evaluation	×	×	×	×	×	×	×
Cell-mediated immunity							
in vitro	×			×			×
in vivo	×						×

CLINICAL EVALUATION

This was based on the following. (1) Patient's own evaluation of pain and morning stiffness. (2) Doctor's evaluation of general weakness and tiredness, fever, hepatomegaly and splenomegaly, and new joint involvement. (3) Physiotherapists measured grip strength in both hands with a sphygmomanometer and an automatic writer, and range of flexion and extension at both wrists and knees. (4) Overall clinical assessment by doctor. Parameters 1 to 3 were graded 0–5; parameter 4, −2 to +2.

LABORATORY INVESTIGATIONS

Biochemical analysis

Erythrocyte sedimentation rate, haemoglobin concentration, white blood count and differential, and platelet counts on peripheral blood; and urine analysis. In the final analysis only the erythrocyte sedimentation rate, graded 0–5, was used. Other tests performed before the trial were blood serum electrophoresis; serum creatinine, alkaline phosphatase, and transaminases; electrocardiogram and chest *x*-ray.

Percentage and absolute number of different lymphocyte populations

These were estimated by the method of Frøland and Natvig (1973). T lymphocytes were identified by their receptors for sheep erythrocytes; B lymphocytes by their membrane-bound Ig detected by immunofluorescence staining with fluorescein isothiocyanate-labelled rabbit antiserum raised against $F(ab')_2$ and Fc-receptor-bearing lymphoid cells by rosette formation with erythrocytes sensitised with human Ig.

Cell-mediated immunity in vitro

This was studied by lymphocyte transformation after stimulation with a panel of unspecific and specific mitogens as previously described (Høyeraal *et al.*, 1975). The unspecific mitogens used were phytohaemagglutinin, pokeweed mitogen, and concanavalin A. The specific antigens used were preparations of *Candida albicans* and purified derivatives of tuberculin. Mitomycin C-treated allogeneic cells in mixed lymphocyte culture tests were also used.

Cell-mediated immunity in vivo

This was studied by delayed skin hypersensitivity as reported elsewhere (Høyeraal *et al.*, 1975).

microbial antigens were injected intradermally in two dilutions. The mean of the maximal diameters (length and breadth) of erythema and induration after 24 and 48 hours were used. Measurements were made after 4 hours to exclude possible Arthus-like reactions.

STATISTICS

A two-sided Wilcoxon test for 2 samples was used for each comparison (Diem, 1962). The test for 2 samples rather than the test for paired differences was used due to unequal numbers in the final evaluation of the two groups. A nonparametrical test was chosen because there was no reason to believe that the factors measured were parametrically distributed. The median of the values of the differences before and after treatment were calculated. $P < 0.05$, indicating statistical significance.

Results

One patient in the placebo group was excluded from the trial after 3 months because of severe exacerbation of the disease. There was no significant difference between the two groups in the change in pain and morning stiffness as assessed by the patients (Table 2), nor with regard to the objective evaluation of doctors and physiotherapists, nor with the erythrocyte sedimentation rate. Even when calculating the maximal and minimal rank sum in cases where 2 values of differences were equal, the differences were not statistically significant. Thus, treatment of JRA patients with transfer factor was not found to improve or worsen their clinical condition significantly.

In the group treated with transfer factor there was a significantly greater reduction in the percentage of T lymphocytes than in the placebo group (Table 3), but the significance was marginal. A somewhat greater reduction in absolute number of T lymphocytes also occurred in the transfer factor group compared to the placebo group, but this difference was not significant. No significant difference was found between the two groups with regard to alteration in the absolute number of leucocyte or lymphocyte counts in peripheral blood or in the percentage or absolute number of B lymphocytes and Fc-receptor bearing lymphoid cells (Table 3).

Furthermore, the changes in cell-mediated immunity, estimated by tests *in vitro* (Table 4) and *in vivo* (Table 5), did not differ significantly between the two groups. Similar observations were made when diameters of erythema rather than induration were used. As a result of randomisation of the patients into two groups, only 2 patients in the transfer factor-treated group had been tested before this trial, whereas 7 in the placebo group had been, giving a one-sided value of $P=0.04$ by the Fisher-Irwin test for 2-by-2 tables. Technical difficulties with some *in vitro* cellular studies reduced the number of patients somewhat (Tables 3, 4).

No side effects could be related to the treatment with transfer factor. The results indicate that transfer factor injections caused neither a flare-up nor a worsening of the disease, nor clinical improvement of the arthritis.

Table 2 *Statistical evaluation of clinical course in a double-blind 6-month parallel group trial of the effect of transfer factor on JRA patients*

Criterion	*Transfer factor (n=12)*	*Placebo (n=11)*	*R**
Pain and morning stiffness	−0·75	−0·25	165
Objective, by doctor	−0·25	0	154
Objective, by physiotherapist	0·05	−0·15	147
Overall clinical assessment	0	0	152
Erythrocyte sedimentation rate	0·25	0	150

Note: Median values are given of differences obtained when clinical evaluation grades before the trial were subtracted from grades after the trial.
*Sum of ranks for transfer factor-treated patients, Wilcoxon test for 2 samples. Critical value of R, indicating that transfer factor was significantly ($P<0.05$) better than placebo, is 180, and if no difference $R=150$.

Discussion

The preparations of transfer factor used were not found to be of any significant therapeutic value for

Table 3 *Statistical evaluation of leucocytes and lymphocytes in the trial*

Cells	*Transfer factor* (n=12)*	*Placebo* (n=11)*	*R†*	*‡*	*§*
Leucocytes	−250 (8)	−350 (8)	63	49	87
Lymphocytes	−324 (8)	−1382 (8)	57	49	87
T lymphocytes (%)	−28 (11)	5 (8)	136	85	135
Absolute no. of T lymphocytes	−1319 (7)	−82 (5)	55	33	58
B lymphocytes (%)	−3 (10)	−2 (8)	98	72	118
Absolute no. of B lymphocytes	−26 (7)	−147 (6)	43	34	64
Fc-bearing lymphoid cells (%)	−9 (9)	2 (9)	96	62	109
Absolute no. of Fc-bearing lymphoid cells	222 (5)	−101 (6)	20	18	42

*Median values of differences are given when values before the trial were subtracted from values after the trial; number of patients in parentheses.
†Sum of ranks (Wilcoxon test for 2 samples).
‡Lower and § upper critical ($P<0.05$) value of R.

Table 4 *Statistical evaluation of* in vitro *studies of cell-mediated immunity*

Mitogen/antigen	*Transfer factor* (n=12)*	*Placebo* (n=11)*	*R†*	*‡*	*§*
Phytohaemagglutinin	−73 (7)	44 (10)	62	42	84
Pokeweed mitogen	− 7 (11)	−90 (11)	105	96	157
Concanavalin A	−86 (9)	−77 (11)	95	68	121
Candida albicans	−13 (5)	−18 (8)	38	21	49
PPD	− 3 (5)	3 (8)	38	21	49
Allogeneic cells**	1·1 (5)	1·9 (8)	43	21	49

*Median values given as in Table 3.
†Sum of ranks (Wilcoxon test for 2 samples).
‡Lower and § upper critical ($P<0·05$) value of R.
**Treated with mitomycin C.

Table 5 *Statistical evaluation of delayed skin hypersensitivity estimated by induration diameters*

Antigen	*Dilution or concentration*	*Transfer factor* (n=12)*	*Placebo* (n=11)*	*†*
Candida albicans	1:100	0·5	0	139
	1:10	5	8	162
Streptokinase/ streptodornase	0·05 SK/l	0	0	145
	0·4 SK/l	0	4	153
Mumps virus	1:10	0	1	141
	Undiluted	−1	0	159
Brucella abortus	2×10^3 bact/l	0	0	156
Bang's bacillus	2×10^4 bact/l	4·5	−2	125
PPD	0·03 TU/l	0	0	142
	0·3 TU/l	0	0	135

*Median values given as in Tables 3 and 4.
†Sum of ranks for transfer factor-treated patients. The critical ($P<0·05$) values of R are 111 and 177.

the JRA patients, as assessed by several criteria of disease activity and immunological function. On the other hand, this trial did not make the patients worse clinically. These findings are in accordance with a double-blind 12-week crossover trial in 6 adults with rheumatoid arthritis (Maini *et al.*, 1976), and with an open trial of 8 patients with JRA (Grøhn *et al.*, 1976). In rheumatic diseases with a natural fluctuating course, a longer period (6 months) and a parallel group study are considered more convenient for documenting toxicity and for demonstrating possible specific activity (Hill, 1969; Amor *et al.*, 1974; Frøland *et al.*, 1974; Grøhn *et al.*, 1976; Maini *et al.*, 1976).

As there are still no uniformly accepted diagnostic criteria of JRA and no simple ways of estimating disease activity (Hill, 1969; McCarty, 1972; Amor *et al.*, 1974; Levy and Dick, 1975), a panel of subjective and objective criteria was used. Extensive planning before the trial, the help of statisticians, and regular discussions by the team of investigators during the trial were valuable.

In a study with so many variables, random allocation and wholly unbiased observations are an absolute necessity. The significance of the greater reduction of the percentage of T lymphocytes in the peripheral blood from transfer factor-treated patients than from that of placebo-treated patients is difficult to explain. When performing several statistical analyses on material, however, some reach statistical significance by chance.

No significant potentiation of cell-mediated immunity was found in the patients treated with transfer factor by comparison with the reactivity observed in the placebo group. This is in contrast to the observations made in the open pilot study (Frøland *et al.*, 1974; Kåss *et al.*, 1974; Natvig *et al.*, 1976). The immunopotentiation in those 3 patients may have been due to sensitisation by repeated skin testing (Grøhn *et al.*, 1976; Maini *et al.*, 1976). In order to eliminate this possibility in this study, skin testing was only performed before or after completing the trial. This discrepancy between the results obtained in the pilot and the present study may be due to the great difference in frequency of previous testing in patients in the two groups. More than half of the patients in the placebo group had had skin tests, whereas only 1 out of 6 of the transfer factor-treated patients had.

In conclusion, the transfer factor preparations from healthy blood donors were of no therapeutic value for these JRA patients. We have now stopped using such preparations in the treatment of JRA. The nature of a possible antigen(s) involved in the pathogenesis of JRA is(are) still unknown. Thus, more specific or purified preparations of transfer factor seem to be required if further trials are conducted in patients with JRA.

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