

TRANSFER FACTOR IN THE TREATMENT OF CHRONIC MUCOCUTANEOUS CANDIDIASIS: A CONTROLLED STUDY

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Abstract. A controlled cross-over study with transfer factor was carried out on 7 patients suffering from chronic mucocutaneous candidiasis. Only one patient showed clinical improvement, which started during a period of pretreatment with 5-fluorocytosine given orally 14 days before the patient entered this trial. No conversion to a positive skin test with *Candida* antigen or PPD was demonstrated following TF in the 6 patients who were anergic to either of these antigens. Variations in the cell-mediated immune response as revealed by lymphocyte transformation were observed in most patients, especially when studied over a long period of time. However, no pronounced efficacy of TF vis-à-vis placebo in normalizing the cell-mediated immune response could be demonstrated in the 5 patients who completed the clinical trial. Systemic side effects were not observed.

Key words: Transfer factor; Candidiasis; Controlled study

Chronic mucocutaneous candidiasis (CMC) is a rare condition which has proved to be genetically and immunologically heterogeneous (10, 19, 21). It has a chronic course and most remedies produce at best a temporary remission (10). A low molecular weight fraction of leukocytes, called transfer factor, has been administered to several patients in recent therapeutic trials (ref., see 12). Many of these studies are difficult to evaluate, however, as they involve only a single patient or a very small number of patients and are inadequately controlled. Most published studies report positive results, while negative results may not have been published to the same extent.

In a controlled cross-over clinical trial with transfer factor in 7 patients with CMC we observed clinical improvement in only one patient.

MATERIALS AND METHODS

Preparation of transfer factor and placebo. Transfer factor (TF) was prepared from leukocytes according to the

method described by Reymond and Grob (15). Buffy coats from 115 unselected Scandinavian donors of 400 ml of blood with no detectable hepatitis B surface antigen (HBsAg) by radioimmunoassay, were used for the preparation of one batch. Each ampoule contained transfer factor equivalent to a dialysate of 1×10^9 leukocytes. The placebo preparation consisted of sodium chloride coloured with riboflavin in order to disguise the contents of the ampoules. The placebo and transfer factor preparations were kept freeze-dried at -20°C . Tests of pyrogenicity and sterility as well as safety according to the European Pharmacopoeia were performed on the TF preparation and placebo.

Design of trial. The aim was to perform a controlled double-blind cross-over study. The ampoules were coded, but since local reactions usually appeared at the injection site when TF was given, the clinical part of the study was not done blind. The laboratory analyses were done blind, however.

Each of the 7 patients received 10 ampoules of transfer factor and placebo, respectively, which were labelled as either A or B when randomized according to a scheme of two groups of four. Each patient received preparation A during the first treatment period after which at least three consecutive *in vitro* analyses of cell-mediated immunity had to be subnormal before starting the second period of treatment using preparation B. This procedure was followed in order to avoid any possible carry-over effect when using transfer factor and involved an interruption of at least one month between the treatments with preparations A and B.

Patients. Seven patients with chronic mucocutaneous candidiasis were studied (Table I). There were 2 females and 5 males with a mean age of 17 years (range 4-29). In all but one the onset occurred before 5 years of age. *C. albicans* was cultured from all oral, facial and paronychia lesions. Earlier topical treatments comprised nystatin, gentian violet, natamycin, amphotericin B and clotrimazole, with which no effect or only a slight temporary one was noted. Parenteral iron and oral zinc sulphate had been given in some cases without any effect. One of the patients (No. 1) was treated with 5-fluorocytosine orally for 2 weeks immediately before entering the cross-over study. This patient had previously received seven ampoules of TF in a pilot study, without any clinical effect.

Two patients had endocrine diseases and belonged to group 3 according to the classification proposed by Higgs

Table I. The clinical condition of the seven patients with chronic mucocutaneous candidiasis

Patient no.	Sex	Age (years)	Age at onset (years)	Localization of candida lesions			
				Stomatitis + genital lesions	Paronychia	Scalp + facial skin lesions	Other diseases
1	♂	11	3	+	+	+	---
2	♀	23	4	+	+	0	Oesophageal stricture
3	♂	29	8	+	+	0	---
4	♂	4	4/12	+	+	+	---
5	♂	22	2/12	+	+	+	Recurrent upper respiratory infections
6	♂	14	2	+	+	0	Hypoparathyroidism
7	♀	12	1	+	+	0	Alopecia areata Adrenocortical insuff. Hypoparathyroidism Pernicious anaemia Chronic keratitis

& Wells (7). One of them (No. 6) had an elder brother (without candidiasis) who died at the age of 12 from complications of hypoparathyroidism. The other patient (No. 7) has a brother who also suffers from chronic mucocutaneous candidiasis as well as hypoparathyroidism, hypertension, pernicious anemia and vitiligo, but is not included in this study. As none of the patients in this trial had granulomatous lesions, the other 5 cases would belong to group 1 according to the above classification, although only 2 of them are closely related (No. 2 and 3 are siblings). Serum immunoglobulin levels were normal in all except one patient (No. 5) who had persistently reduced levels of IgG and IgA (IgG 5.5; IgA 0.6 g/l). Different patterns of disturbed cell-mediated immunity were found in the 7 patients (Table II).

Dosage of TF and placebo. The content of one ampoule, diluted with 1–3 ml normal saline, was injected subcutaneously every seventh day. Before each injection a test dose of about one-tenth of the content of the ampoule was given subcutaneously to preclude strong immediate side effects. Body temperature and pulse rate were recorded before and after each injection.

Follow-up tests. A skin test with *Candida albicans* extract (Hollister-Stier, 1/100) and/or purified protein de-

rivative (PPD, 2 TU) was read after 48 hours and laboratory examinations were performed at least once shortly before the trial and then repeated three times or more during each treatment period and in the interval between them. These examinations included hemoglobin; white blood cell count and a differential count; platelets; circulating T and B lymphocytes (8), determined as percentage of E-rosette forming and surface immunoglobulin positive lymphocytes, respectively; in vitro lymphocyte transformation (13) using phytohemagglutinin (PHA), concanavalin A (Con-A), pokeweed mitogen (PWM), *Candida* antigen, PPD and serum anti-candida antibodies. HBsAg in serum was searched for before the trial and at the end of each treatment period.

RESULTS

Clinical improvement was noted in only one patient (No. 1), in whom the skin lesions started to heal during a pretreatment period with 5-fluorocytosine given orally in a dose of 200 mg per kg body weight daily for 2 weeks. Immediately after this premedica-

Table II. Immunological data on the seven patients before entering the trial

Patient no.	T-lymphocytes ^a	Lymphocyte stimulation		Skin test <i>C. albicans</i>
		PHA	<i>C. albicans</i>	
1	Normal	Normal	Neg.	Neg.
2	Normal	Normal	Neg.	Pos.
3	Normal	Normal	Neg.	Pos.
4	Normal	Normal	Normal	Neg.
5	Normal	Normal	Neg.	Neg.
6	Normal	Subnormal	Normal	Slightly pos. (5×6 mm)
7	Subnormal	Normal	Neg.	Neg.

^a Percentage of E-rosette-forming lymphocytes.

tion the patient entered the present trial and received transfer factor once a week. The healing of the skin lesions progressed during this time and the face completely healed before the end of the period of treatment with TF. The patient was then withdrawn from the study as it was judged unethical to interrupt the therapy with transfer factor. Since then the patient has received TF injections regularly every month for 2½ years. He is still free from facial lesions and the paronychia is also healed. The oral candidiasis is still present, but the mucous membranes are less reddened and swollen and he has considerably milder symptoms now than before this treatment regimen was started. In the remaining 6 patients the lesions on the oral mucous membranes, skin and the paronychia infections remained essentially unchanged during the cross-over study.

The immunological investigations did not show a complete conversion from negative to positive response to the test panel of antigens and mitogens used during the time the clinical trial was in progress. In several patients, however, variations in the tests of cell-mediated immunity were demonstrated. Transient weak responses to *Candida* antigen in the lymphocyte transformation test were observed in 4 patients—in 2 of them (Nos. 3 and 4) during both treatment periods and in 2 others (Nos. 1 and 2) during treatment with transfer factor. One patient (No. 3) converted once to a positive reaction to PPD when skin-tested during the treatment period with placebo. On all test occasions previously and afterwards he has been negative.

One patient (No. 6), who 4 years earlier had had a depressed cell-mediated immune response in the lymphocyte transformation test and delayed hypersensitivity test, showed a gradual change to a normal response during the progress of the trial. The patient who had previously been anergic to *Candida* antigen in the skin test became slightly positive (5×6 mm) just prior to entering this trial. At the end of the first treatment period, which appeared to be with transfer factor, the positive reaction increased to 96×165 mm. During the treatment with TF the capacity for lymphocyte stimulation with PHA also increased, from a subnormal response shortly before the trial, to a normal response. This patient was the only one who had an enhanced production of serum antibodies against *Candida* antigen during the progress of the study. Since the cell-mediated immunity was restored, the criterion of three consecutive negative results for cell-mediated immuni-

ty before the change to the second treatment period with placebo could not be fulfilled. Consequently, this patient had to be withdrawn from the study after the TF therapy.

The most frequent side effect was a reddened, slightly painful infiltration at the injection site, which subsided in a day or two. No fever, allergic or anaphylactic reactions occurred. Blood cell dyscrasias were not recorded. Tests for HBsAg remained negative in all patients.

DISCUSSION

The aim of this study was to examine the clinical effect of TF alone. Thus, pretreatment with systemic anti-fungal agents was avoided. In 6 patients receiving TF therapy alone no clear clinical benefit could be demonstrated. One additional patient was treated with TF, however, just after a short course of an oral anti-candidal agent, 5-fluorocytosine. The clinical improvement achieved with this agent progressed during TF therapy and, 2 years later, he is still free from skin lesions. In a pilot study one year earlier this patient received TF prepared from donors who had positive skin reactions to *Candida* antigen. A total of seven ampoules, each of which contained about the same amount of TF as used in this study, were injected subcutaneously over a period of one month. No clinical improvement was observed. Conversion to a positive response to PHA in a lymphocyte transformation test was noted, however, and was maintained during this clinical trial.

In the immunological investigations no distinct conversion to a positive response to mitogens *in vitro* or to skin test antigens *in vivo* could be observed during the progress of the present trial. One patient (No. 6) showed a slight positive response to *Candida* antigen by the delayed hypersensitivity skin test just before the first period of treatment during which TF was given. During the progress of the trial this responsiveness gradually improved. The increase in the reactivity which had occurred since previous negative tests 4 years earlier might have been spontaneous or induced by earlier skin tests. The remaining 6 patients showed an unaltered response to antigens and mitogens used in the immunological investigation, although transient variations, especially in the lymphocyte transformation test, were observed.

The results of the immunological examinations support the concept of a disturbed cell-mediated immunity in patients with CMC although it may be secondary to an unknown primary event (3, 11, 19). Stimulation of the cell-mediated immune mechanism with TF represents an effort to overcome the postulated immune deficiency (12). Enhancement of resistance to *Candida* infection by TF alone in patients with CMC has been claimed by some workers (1, 2, 6, 17, 18, 20). These results contrast with those of the present study carried out under controlled conditions in 7 cases of CMC. The divergent results could be due to differences in the selection of patients, of TF donors, the method of preparing TF, inadequate dosage, the presence of blocking factors (19) in the recipients, or a lack of TF effect.

Patients with CMC constitute an extremely heterogeneous group, and it is essential that they should be carefully evaluated by immunological methods (11, 19). Our patients seem to be a representative sample of early onset CMC, although none showed the more severe granulomatous involvement of the skin. The TF donors were unselected, but most healthy Scandinavian residents have shown a positive response to *C. albicans* antigen in the lymphocyte transformation test (5). It is therefore conceivable that the donors were capable of a cell-mediated immune response to *C. albicans*. Our TF was prepared by a standard technique. Unfortunately, it is still difficult to calibrate in a reproducible manner the activity of TF *in vitro*. Besides, the chemical nature of TF is unknown (12).

The clinical change in the single patient who improved appeared during the pretreatment period with 5-fluorocytosine. Since then the patient has received TF without recurrence of the skin lesions. As a rule, systemic or topical antimycotic treatment of early onset CMC produces only temporary improvement (10). Initial reduction of the antigen load is a well-known principle in immunotherapy of advanced malignant tumours. Kirkpatrick (11) has suggested that patients with CMC starting before the age of 5 years should receive this combined therapy, since TF alone might be insufficient. Our limited experience of one case is consistent with Kirkpatrick's suggestions, as are several case reports (4, 9, 14, 16, 18). It might be worthwhile to try this approach in our other patients who did not respond to TF alone.

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