TRANSFER FACTOR THERAPY IN A PATIENT WITH CHRONIC VAGINAL CANDIDIASIS

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Summary
A 32-year-old patient with chronic vaginal candidiasis was treated with 8 units of Transfer Factor (TF). Smears and cultures for Candida albicans became negative. Vaginal discharge and itching ceased, and the clinical condition of the vagina improved. Formerly negative delayed type skin tests with candidin converted to positive and Candida lymphocyte stimulation became measurable, suggesting that a defective cellular immunity towards Candida aas restored by TF. Candidiasis reappeared a few months after the last dose of TF when measurable cellular immunity towards candidin was still present. The condition responded rapidly to a local treatment with 5-fluoro-cytosin.

CHRONIC or recurrent candidiasis may be associated with various defects of cell mediated immunity (CMI) (Blaker et al., 1973; Buckley et al., 1968; Chilgren et al., 1967; Higgs and Wells, 1973; Kirkpatrick et al., 1971; Montes et al., 1972; Paterson et al., 1971).

Transfer Factor (TF), a dialysable extract of human leucocytes, is thought to transfer CMI from one individual to another (see review by Lawrence, 1969). Many clinical trials have shown that CMI deficiencies can be corrected by TF, leading in at least some patients to clinically beneficial effects (Grob et al., 1973; also see review by Hitzig and Grob, 1974). This paper describes a patient with chronic vaginitis due to Candida albicans infection combined with immune defects who was treated with TF.

MATERIALS AND METHODS
Production of TF
Heparinized blood (500 ml.) from normal blood bank donors was collected in plastic bags and centrifuged at 4000 g for 5 minutes. Theuffy coat (leucocyte rich fraction) was removed by careful compression of the bag. Buffy coats from 10 to 20 donors were pooled, resuspended in an equal amount of saline, and slowly frozen ten times at -20 °C. and quickly thawed in a waterbath at 37 °C. This material was passed through a “Sartorius” ultrafiltration cell (membrane SM 121–36) or through an “Amicon” ultrafiltration cell (membrane PM-10), allowing only the passage of molecules with molecular weights below 10,000. The ultrafiltrate was again filtered through a “Millipore” membrane (pore
size 0.3μ), immediately poured into ampoules and lyophilized. The lyophilized substance was redissolved in sterile water and injected subcutaneously as a hypertonic solution (600 to 800 mosm.). One unit of TF was defined as the dialyzed and lyophilized extract of 1 to 2 × 10^9 leucocytes (see Grob et al., 1974).

**Schedule of treatment**

One unit of TF (test dose) was given subcutaneously in six portions within 6 to 8 hours (day 1), followed by 3 units of TF one month later, and again by 5 units three weeks later.

**Follow-up tests**

The following tests were performed before treatment was started, between further TF injections and one, four and seven months after the last dose.

(a) Skin tests of the delayed type were done, according to the WHO recommendations (WHO/OMS, 1971) with two preparations of candidin (Dermatophytin "O" 1 : 100, Hollister-Stier and Oidiomycin 1 : 10 from our mycological laboratory), varidase (streptokinase 4 units, streptodornase 1 unit), mumps skin test antigen, purified protein derivative (PPD) 1 : 1000, Trychophytin 1 : 30, coccidioidin 1 : 100, and *Staphylococcus aureus* antigen.

(b) DNA synthesis in lymphocytes was estimated by measuring thymidine uptake after stimulation of the recipient's peripheral lymphocytes with candidin and phytohemagglutinin (PHA) using the technique of Bach and Voynow (1966) as modified by Bodmer and Hitzig (1971). An increase of more than 2.5 fold in cpm counts when comparing stimulated and non-stimulated cells was regarded as evidence of enhanced thymidine uptake and DNA synthesis.

(c) Immunoglobulin concentration and β1A (a measureable metabolic product of the third component of complement) were measured by radial immunodiffusion.

(d) Bacteriological and fungal examinations of smears and cultures were made according to current techniques.

**Case History**

The patient was born in 1942 and was fit until 1968, when she developed vaginal discharge and itching. The discharge continued to occur over five years with exacerbations after each menstrual period. Clinically, the patient represented a chronic form of vaginitis due to *Candida* infection. The vaginal mucosa was covered with scaling confluent plaques of various sizes (diameter up to 10 mm.). Repeated bacteriological and mycological examinations (smears and cultures) always revealed the presence of *Candida albicans*. The patient had never taken oral contraceptives. A glucose tolerance was normal, and the leucocyte count was 10,300 per cu./mm., with 61 per cent neutrophils. There was no evidence of hypoparathyroidism, Addison's disease or a thymoma.

The patient was treated with nystatin pessaries and tablets from 3rd to 24th October 1972, Nifuratel tablets and pessaries from 24th October to 10th November 1972, local applications of 1 per cent econazole (1/-2,4-dichloro-β-(p-chlorobenzylxylo)-phenethyl/-imidazole nitrates) from 11th November 1972 to 9th to 13th February 1973, and miconazolnitrate tablets from 14th February to 16th April 1973 with only slight and transient improvement of symptoms. In April 1973 the decision was made to commence with TF therapy. The immunological tests seemed to justify such treatment: delayed skin reactions (type IV reaction according to Coombs and Gell, 1968) were repeatedly negative for the two *Candida* extracts Dermatophytin "O" and Oidiomycin, as well as for varidase and coccidioidin, but strongly positive with PPD (erythema/infiltration=40/12 mm.) mumps (17/10 mm.) and *Staphylococcus aureus* antigen (67/17 mm.). A wheal and flare reaction occurred with candidin, but disappeared within 30 minutes (Type I reaction). Thymidine uptake was negative after stimulation with candidin, but normal with PHA. The immunoglobulins (1gA 2.8 mg./ml., 1gG 12.0 mg./ml., and 1gM 2.3 mg./ml.) as well as β2A-globulin (120 mg. per cent) were in the normal range. Antibodies against poliomyelitis, measles, rubella, viruses and *Candida albicans* were detectable. These findings led to a diagnosis of vaginal candidiasis with a selective defect of CM1 for *Candida* antigens.

**Results of Therapy**

No clinical effects were seen after the first dose of TF, but the formerly negative skin tests with candidin became weakly positive (Derma-
tophysitin “0”: erythema/infiltration = 9/3 mm.; Oidiomycin 9/6 mm.; Table I.) Thymidine uptake after lymphocyte stimulation with candidin became positive and the PHA stimulation, which already had been normal before, increased markedly.

The second injection was followed by a regression of local pain and itching, and the discharges became less abundant. The skin tests with candidin became strongly positive (Dermatophysitin “0” 38/15 mm.; Oidiomycin 28/14 mm.). For the first time in six years Candida could not be found in the smears and cultures made from discharge and from vaginal scrapings. After the third injection of TF, vaginal discharge practically ceased, itching disappeared completely and the vaginal mucosa appeared normal with only a few isolated white plaques. All TF injections were accompanied by severe pain around the injection site lasting for several hours. The skin test with candidin as well as the lymphocyte stimulation were still positive at two and seven months after the last injection of TF.

Two months after the last injection of TF the smears were still negative for Candida, but the fungus was again isolated in cultures. Five months after the last dose of TF, pruritus

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<td></td>
<td><strong>TF</strong></td>
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<td>Vaginal discharge</td>
<td>+++</td>
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<tr>
<td>Involvement of vaginal epithelium</td>
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<tr>
<td>Itching</td>
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<tr>
<td>Candida</td>
<td>+++</td>
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<tr>
<td>in Smears</td>
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<tr>
<td>in Cultures</td>
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<tr>
<td>Skin test with candidin (diameter of erythema in mm./diameter of infiltration in mm.)</td>
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<tr>
<td>Dermatophysitin “0”</td>
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<tr>
<td>Oidiomycin</td>
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<tr>
<td>PHA</td>
<td>(272)</td>
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<td>Candidin</td>
<td>(1.6)</td>
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<td>IgA (0.6–4.9)*</td>
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<td>IgG (7.1–15.4)</td>
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<td>IgM (0.4–2.0)</td>
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<td>β1A-globulin (mg./100 ml.)</td>
<td>(110–190)</td>
<td>120</td>
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* = Normal range.
reappeared transiently despite a continuously positive skin test and positive *Candida* lymphocyte stimulation. However, only ten days’ further treatment with two 500 mg. vaginal tablets of 5-fluoro-cytosin daily was needed before all symptoms regressed.

The immunoglobulin levels remained normal during the whole course of treatment, and the concentration of β1A-globulin dropped only slightly for a short time after the second dose of TF.

**DISCUSSION**

According to the literature, 36 patients with chronic mucocutaneous candidiasis and defects of CMI have so far been treated with TF (Bläker *et al.*, 1973; Griscelli *et al.*, 1973; Hitzig *et al.*, 1972; Hobbs and Valdimarsson, 1973; Kirkpatrick *et al.*, 1972; Pabst and Swanson, 1972; Rich *et al.*, 1972; Rocklin *et al.*, 1970; Schulkind *et al.*, 1972; Valdimarsson *et al.*, 1972). In 27 of these patients one or more immunological parameters of CMI converted from negative to positive. In 19 individuals at least transitory clinical improvement was observed. Some of the good results were obtained with the simultaneous use of TF and antimycotics, but in most patients antimycotic drugs alone had been used without success before TF therapy was started. Beneficial

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<td>↑ units</td>
<td>↑ 5 units</td>
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<td>38/15</td>
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clinical effects as well as immune conversions had often been weak and of short duration after the first dose of TF, but became more pronounced and longer lasting when therapy was continued (Bläker et al., 1973; Grob et al., 1974; Valdimarsson et al., 1972; and others). A similar phenomenon was observed in our patient, to our knowledge the first for chronic vaginitis treated with TF. Conversion of the candidin skin tests and of the lymphocyte stimulation with candidin suggest restoration of a formally defective CMI towards this antigen. Disappearance of Candida in the cultures and smears further suggests that TF treatment was effective. It is difficult to judge whether the good and lasting effect of 5-fluoro-cytosin was aided by the reconstitution of CMI by TF or not.

In the patient described, the pain at the TF injection site lasted more than 12 hours, and was severe, a phenomenon which so far occurred only in 2 of 45 patients treated with a total of 409 units of TF produced in our laboratory. Slight pain lasting 1 to 2 hours was observed in about half of these 45 patients. The pain was probably due to the hypertonic solution of TF used for injection (Grob et al., 1974).

Others have reported more serious complications such as haemolytic anaemia (1 patient, Ballow et al., 1973), polyclonal gammopathy (1 patient, Gelfand et al., 1973), nephrotic syndrome (1 patient) and malignancies (2 patients) (Spitler, personal communication, 1973) but their causal relationship to TF is uncertain as they occur in other immunodeficiency diseases. According to the literature, 233 patients had been treated with TF up to the end of 1973 (reviewed in Grob et al., 1974). In addition to candidiasis, good results have been obtained in at least some patients with disseminated coccidiomycosis (five patients; Graybill et al., 1973a), tuberculosis (two patients; Graybill et al., 1973b; Whitcomb and Rocklin, 1973), interstitial pneumonia (one patient; Dupree et al., 1972), Wiscott Aldrich syndrome (Griscelli et al., 1973; Hitizig et al., 1972; Levin et al., 1970; Spitler et al., 1971 and 1972a; Wybran et al., 1973). Ataxia telangiectasia (Griscelli et al., 1973; Grob et al., 1974), several forms of other combined immunodeficiency diseases (Hitizig et al., 1972; Lawlor et al., 1973; Valdimarsson et al., 1974; Wybran et al., 1973), nasopharyngeal carcinoma (Goldenberg and Brandes, 1972), and melanoma (Brandes et al., 1971; Morse et al., 1973; Spitler et al., 1972b; Grob et al., 1974.)

It is widely accepted that TF preparations contain several immunologically and biologically active substances. Their nature and structure is not yet certain, but TF activity seems to be bound to molecules with molecular weights below 10000. It is resistant to DNAse, RNAse and trypsin and is destroyed by pronase; TF might be a complex of a small polypeptide and an oligoribonucleotide (Lawrence, 1969 and 1972) and its exact mode of action has yet to be elucidated.

Acknowledgements

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