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Research Article

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# Effect of Transfer Factor on Lymphocyte Function in Anergic Patients

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ABSTRACT Dialyzable transfer factor, obtained from frozen-thawed peripheral blood leukocytes from a single donor, was given to five anergic patients with chronic mucocutaneous candidiasis. Studies of immunological responses including delayed cutaneous hypersensitivity, in vitro antigen-induced thymidine incorporation, and production of macrophage migration inhibition factor (MIF) were conducted both before and after injection of transfer factor.

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The fifth patient, a 9-yr old boy with an immunologic profile similar to the Nezelof syndrome, did not become skin test-reactive or develop positive responses to the in vitro tests.

These findings suggest that transfer factor acts on the immunocompetent cells that respond to antigens with lymphokine production, but has little, if any, effect on cells that respond to antigens by blastogenesis. The failure to sensitize the subjects with chlorodinitrobenzene illustrates the specificity of the immunologic effects of transfer factor, and implies that it does not function through nonspecific, adjuvant-like mechanisms. Failure of transfer factor to produce positive skin tests or MIF production in a patient with Nezelof's syndrome may be evidence that lymphokine-producing cells are thymus derived.

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## INTRODUCTION

In 1955, Lawrence (1) reported that disrupted leukocytes from skin test-reactive donors could transfer delayed hypersensitivity to skin test-negative subjects. The skin test conversion was specific and long lived. The active component, transfer factor, was subsequently found to be dialyzable and resistant to degradation by trypsin, ribonuclease, or deoxyribonuclease (2). However, its composition and mechanism of action have not been elucidated.

Recently, transfer factor has been employed in treatment of a variety of disorders with disturbed cellular immunity. Patients with the Wiskott-Aldrich syndrome, leprosy, chronic candidiasis, and neoplastic disorders have received transfer factor, and while the results have been variable, some encouraging reports have emerged (2–7).

The purpose of this study was to investigate the effect of transfer factor on immunologic responses of anergic patients with chronic mucocutaneous candidiasis. Cellular immunity was characterized in vivo by intradermal tests and in vitro by lymphocyte transformation and macrophage migration inhibition factor (MIF)[1] production before and after transfer factor. The clinical responses of the recipients were also observed.

[1] *Abbreviations used in this paper:* CDNB, 1-chloro-2,4-dinitrobenzene; MIF, migration inhibition factor; PPD, purified protein derivative; SK-SD, streptokinase-streptodoranse.

## METHODS

*Skin testing.* Each subject was tested with a panel of commercial antigens which included intermediate strength purified protein derivative (PPD), histoplasmin, mumps skin test antigen, trichophytin (1000 pnu/ml),[2] *Candida albicans* extract (Dermatophytin O, 1:100),[2] and streptokinase-streptodornase (SK-SD)[3] diluted to contain 40 U of SK and 10 U of SD/ml. When delayed skin tests with this concentration of SK-SD were negative, the tests were repeated using solutions containing 10 times more antigen. Additional antigenic preparations of *C. albicans* were made by sonication of organisms isolated from cases R. H. and J. A. C. (8), and here the preparations used for skin testing contained 10 or 100 μg of protein/ml. For testing, 0.1 ml doses of antigen were injected intradermally. Cutaneous responses were read at 15 min and 5, 24, and 48 hr. Positive delayed reactions produced 0.5 cm or more of induration.

*Induction of contact allergy.* If skin tests with the panel of naturally encountered antigens suggested anergy, an attempt was made to induce contact allergy with 1-chloro-2,4-dinitrobenzene (CDNB). To screen for pre-existing sensitivity, the subjects were first tested with 100 μg of CDNB in acetone. If this test was negative, a 2000 μg sensitizing dose was applied dropwise to the skin of the medial upper arm. The acetone was evaporated and the area was covered with a Telfa patch for 48 hr. This site was examined regularly for the appearance of a spontaneous flare compatible with induction of contact allergy. Challenge tests with 50 and 100 μg of CDNB were conducted 2 wk later, with the antigens applied to the contralateral arm. The scoring of responses at 24, 48, and 72 hr was essentially the same as that recently described by Catalona, Taylor, Rabson, and Chretien (9), except that biopsies were not done to evaluate grossly negative areas. This schedule of sensitization has been shown by ourselves and others (9) to induce contact allergy in approximately 95% of control subjects.

*Lymphocyte transformation.* The general technique for measurement of antigen-stimulated thymidine incorporation has been described previously (10). Briefly, blood was collected into heparinized (20 U/ml) syringes and the lymphocyte-rich leukocyte fraction was obtained by differential centrifugation and sedimentation. Each culture tube contained $2 \times 10^6$ lymphocytes in 2.0 ml of Eagle's minimum essential medium with penicillin, streptomycin, and either 20% heat-inactivated fetal bovine serum or 20% fresh autologous or homologous plasma. Antigen-stimulated and control cultures were conducted in triplicate or quadruplicate. No patient was receiving drugs known to alter lymphocyte-mediated immunologic responses.

Preliminary studies had shown that peak thymidine incorporation by lymphocytes from skin test-positive subjects occurred on the 5th day of culture, and dose-response experiments indicated that optimal responses were obtained in cultures containing 5 μg/ml of candida extract or 50/12.5 U/ml of SK-SD. Depending upon the number of cells available, cultures contained the optimal concentrations of antigens and other doses ranging from 0.01 to 100 times the optimal doses.

Studies of thymidine incorporation by lymphocytes stimulated with purified phytohemagglutinin,[4] or concanavalin A[5] were harvested on the 3rd day of culture. Mixed leukocyte reactions were done by culturing responding cells with mitomycin-blocked stimulating cells (11) from unrelated, phenotypically disparate donors[6] and were harvested on the 7th day.

Cells were labeled with thymidine-$^3$H 4 hr before harvesting. The cultures were terminated by collecting cells by centrifugation, washing the cells with saline, and precipitating the protein with cold trichloracetic acid. The pellet was washed with cold methanol, digested with sodium hydroxide at 60°C, and dissolved in a toluene-base solvent for liquid scintillation counting.

*Macrophage migration inhibition factor (MIF).* The method of Rocklin, Meyers, and David (12) was employed to study MIF production by lymphocytes from the patients as well as control subjects with either positive or negative skin tests to candida or SK-SD. Lymphocyte-rich leukocyte suspensions were incubated in serum-free minimum essential medium that had been supplemented with glucose, pyruvate, and nonessential amino acids (13), either with or without antigens. Every 24 hr for 3 days the culture fluids were harvested and replaced. Cell-free culture fluids were also collected for control of inhibitory effects of the antigens alone. The supernatants were concentrated fivefold by vacuum dialysis and MIF activity was determined by the "indirect" assay using oil-induced peritoneal exudate cells from guinea pigs. The fields of migration were projected onto a screen and traced and the areas were measured with a planimeter. The per cent inhibition was calculated as suggested by Rocklin et al. (12) and the differences between means were analyzed with the two-tailed *t* test.

*Preparation of transfer factor.* The transfer factor was prepared from cells from one healthy adult donor. Intradermal tests had produced the following diameters of induration at 24 hr: histoplasmin, 4.5 cm with subsequent slough; candida (10 μg/ml), 2.5 cm; SK-SD (40/10 U/ml), 2.0 cm; and mumps, 3.0 cm. There were no responses to PPD or trichophytin. Serologic studies for the hepatitis-associated agent were negative.

A lymphocyte-rich leukocyte suspension was obtained by continuous flow centrifugation with an NCI-IBM cell separator (14). Transfer factor was prepared by the method of Lawrence (2). Cells were lysed by 10 cycles of freezing in dry ice-alcohol and thawing at 37°C. The lysate was then digested with deoxyribonuclease[7] in the presence of magnesium for 1 hr at 37°C. This material was dialyzed against 20 vol of sterile distilled water for 40 hr at 4°C. The dialyzable fraction was lyophilized, then reconstituted with distilled water so that the material from $300 \times 10^6$ lymphocytes was contained in 1 ml. The solution was passed through a Millipore filter, portioned into sterile vials, and stored at −30°C. All steps used sterile glassware and pyrogen-free reagents, and the final product was sterile. In these studies, a "dose" of transfer factor was 2 ml and contained the material from $600 \times 10^6$ lymphocytes. It was given both subcutaneously and into multiple intradermal sites. Skin tests after injections of transfer factor were applied to the contralateral arm.

## RESULTS

*Clinical and immunologic findings before transfer factor.* The initial clinical and immunologic studies of

[2] Hollister-Stier Laboratories, Downers Grove, Ill.
[3] Varidase, Lederle Laboratories, Pearl River, N. Y.
[4] Burroughs Wellcome Co., Research Triangle Park, N. C.
[5] ICN Nutritional Biochemicals Div., Cleveland, Ohio.
[6] H-LA phenotypes were determined by a microcytotoxicity method in the laboratory of Dr. Paul Terasaki.
[7] Worthington Biochemical Corp., Freehold, N. J.

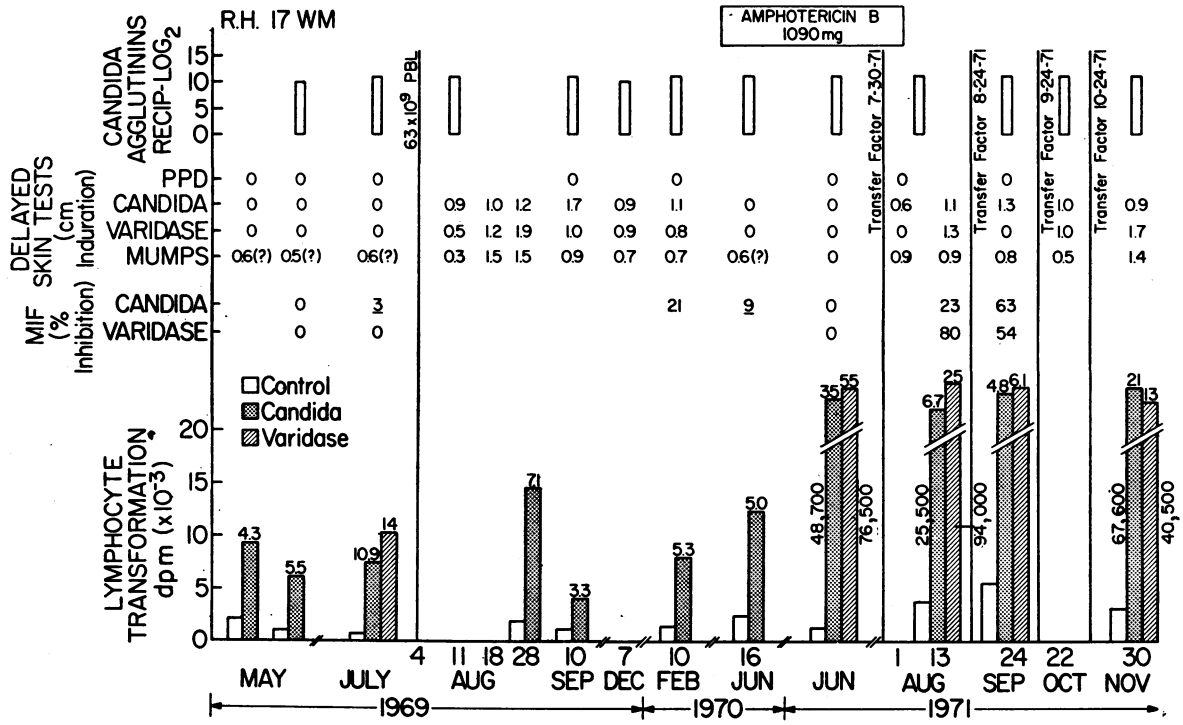


FIGURE 1 Serial studies of lymphocyte transformation, delayed cutaneous hypersensitivity, and MIF production in patient R. H. In all figures the lymphocyte transformation experiments were conducted in 20% autologous plasma. Candida-stimulated cultures contained 5 μg protein/ml of candida extract and Varidase-stimulated cultures contained 50 U of SK and 12.5 U of SD/ml. The ratio of mean dpm in antigen-stimulated cultures to mean dpm in unstimulated cultures (stimulation ratio) is given at the top of each bar. Underlined values for MIF activity represent results in which antigen-stimulated fluids did not produce inhibition that was significantly greater than the control fluids.

three patients (R. H., J. A. C., and D. L.) have been described previously (15). R. H. is a 19-yr old white male who has had extensive candidiasis of the skin, nails, and mucous membranes since age 9 months. Associated disorders included hypoparathyroidism recognized at age 7 yr, and dysplasia of the dental enamel. Treatment with topical medications had produced brief and incomplete remissions. The immunologic studies had revealed nonreactivity to all antigens in the panel, including CDNB. His lymphocytes failed to produce macrophage migration inhibition factor (MIF) when stimulated with candida or SK-SD although both antigens stimulated thymidine incorporation (Fig. 1).

In 1969, the therapeutic potential of immunologic reconstitution was evaluated (16). The patient's father had reactive skin tests to candida, mumps, and SK-SD and was sensitized with CDNB. After transfusion of $63 \times 10^9$ paternal lymphocytes, the patient's skin tests became positive and there was marked clearing of the cutaneous and mucous membrane lesions. Clinical relapse occurred approximately 8 months later and was accompanied by loss of skin test reactivities. In 1970, treatment with clotrimazole (17) produced essentially complete clearing of the oral and cutaneous lesions. Unfortunately, the infections recurred while he was still receiving the drug and increased dosages produced intolerable side effects (nausea, abdominal discomfort, and hypercalcemia), but no clinical benefit.

Because programs directed at correction of the immunologic defect and against the microorganism had each been temporarily beneficial, combined anti-fungal and immunologic treatment was begun in June 1971. Amphotericin B, 1090 mg, was given intravenously during June and July and injections of transfer factor were started in July 1971. There was prompt and complete clearing of all lesions except for the nails, which were avulsed under general anesthesia. The only recurrences have been two episodes of candidiasis on the tongue which followed treatment with antibiotics. These were cleared with amphotericin.

J. A. C. is a white female who was first studied at age 23 yr. She has had candidiasis of the mucous membranes since age 1 month. Cutaneous and nail lesions developed during childhood and have persisted in spite

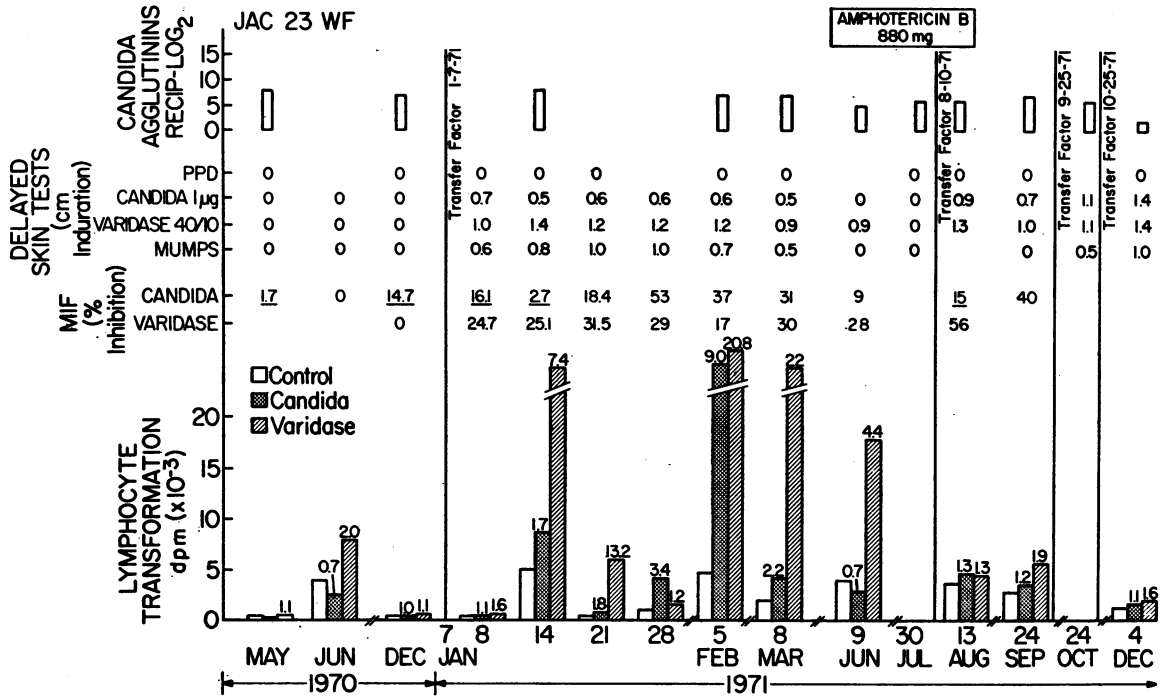


FIGURE 2 Serial studies of MIF production, lymphocyte transformation, and delayed skin tests in patient J. A. C. The culture conditions are the same as Fig. 1. In contrast to the other subjects of this study, this patient had positive lymphocyte transformation responses after the first injection of transfer factor.

of vigorous topical therapy. Symptoms of hypothyroidism appeared at age 21 yr, and the diagnosis was confirmed 2 yr later. Her immunologic studies also revealed anergy to all of the natural antigens in the panel and an attempt at sensitization with CDNB was unsuccessful. The results of serial skin testing and in vitro studies with her peripheral blood lymphocytes are summarized in Fig. 2. Her cells did not respond to antigenic stimulation with increased thymidine incorporation or MIF production.

The first attempt at immunologically directed therapy of this patient was in January 1971, when she was given transfer factor from $600 \times 10^6$ lymphocytes. Although the skin tests to candida, SK-SD, and mumps, became positive, there was no clinical improvement. The combined program of anti-fungal therapy and immunological reconstitution was begun in June 1971 (Fig. 2). The dose of 880 mg of amphotericin B was given intravenously over 10 wk and transfer factor was started 10 August 1971. There was prompt clearing of the cutaneous and mucous membrane lesions, and the nails were avulsed after cultures from the nail folds ceased yielding *C. albicans*. There has been no relapse.

R. V. was first seen at NIH at age 32 yr. At age 3 yr she developed a crusting, pustular dermatitis of the scalp. At age 5 the disorder was diagnosed as candidiasis and during the next 12 yr the lesions progressed to involve the skin of the face, extremities, and torso, as well as the nails and mucous membranes. Diabetes mellitus was found at a routine examination at age 18 yr. At age 24, severe frequency and dysuria prompted a urological evaluation. Cystoscopy disclosed a contracted bladder, a urethral stricture, and severe urethritis and cystitis. Pyelography showed right hydronephrosis and hydroureter. A compensated Coombs-positive hemolytic anemia and episodic leukopenia have been present since 1965. In addition to continuous treatment with topical antifungal agents, she had received intravenous amphotericin B in 1965, 1967, and 1970. Each course had produced marked clinical improvement, but the mucous membrane and cutaneous lesions recurred within a few months. 5-fluorocytosine had also been given, but without benefit.

Upon admission to NIH, the hematocrit was 32% and there was 3.1% reticulocytes. The blood urea nitrogen and creatinine were 19 and 1.1 mg/100 ml, respectively, and the 24 hr creatinine clearance was 24 ml/min. With the exception of diabetes mellitus, there was no endocrinopathy. The anti-nuclear factor was intermittently positive and titers to 1 : 160, the anti-DNA antibody was negative, and the bentonite flocculation titer was 1 : 128. Six lupus erythematosus preparations were negative, and the total serum complement was normal. Immunologic

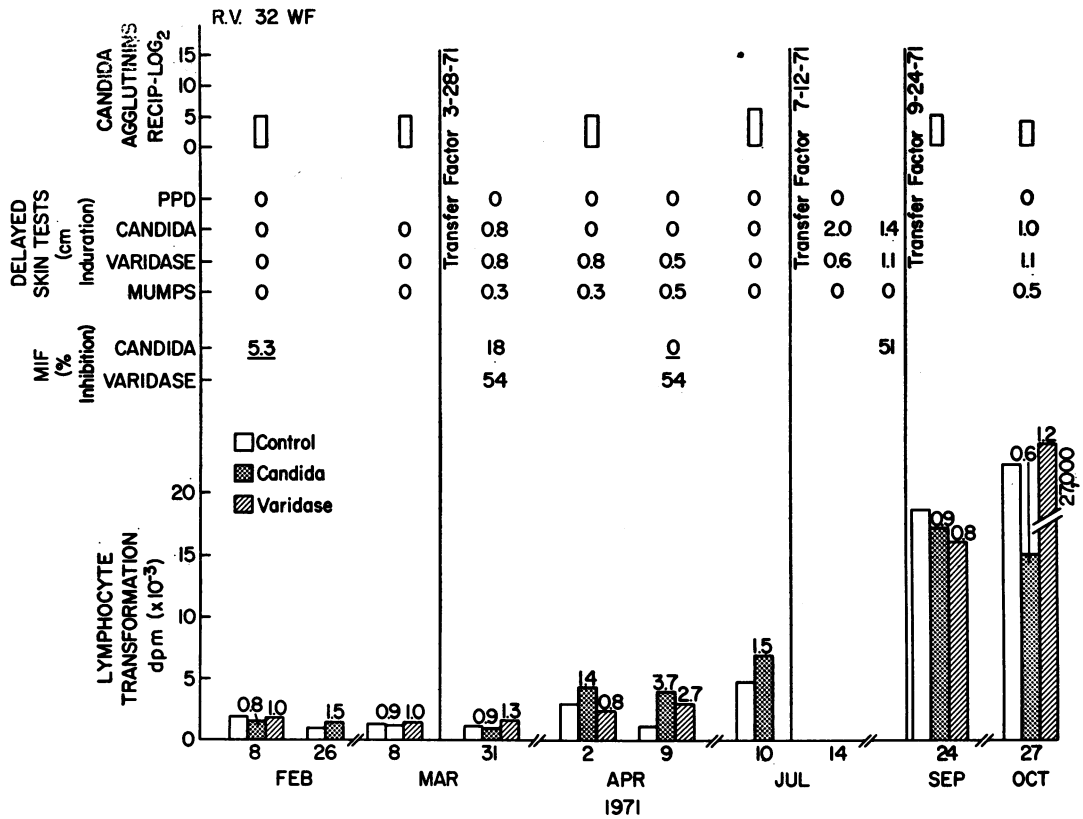


FIGURE 3 Effect of transfer factor on delayed cutaneous hypersensitivity and in vitro lymphocyte responses of patient R. V.

studies disclosed anergy to all the natural antigens in the panel and two attempts at sensitization with CDNB did not induce contact allergy. When exposed to candida or SK-SD in short-term leukocyte cultures, her lymphocytes did not respond with increased thymidine incorporation or MIF production (Fig. 3).

It was felt that the patient's renal status mitigated against further systemic therapy with amphotericin B. A trial of transfer factor was begun in March 1971 (Fig. 3). Although the cutaneous responses to candida and SK-SD became positive, there was only equivocal clearing of the cutaneous lesions. Transfer factor was discontinued in October 1971, when she had a respiratory infection requiring antibiotic therapy and a concommitant exacerbation of the hemolytic process.

D. L. is a 23-yr old male who has had mucocutaneous candidiasis since age 6 months. The most extensive lesions have involved the oral cavity, nails, and skin of the fingers and thorax. This patient had also had repeated staphylococcal infections including pyoderma, subcutaneous abscesses, and lung abscesses. Routine laboratory studies and an extensive survey of his host-defense mechanisms were unremarkable except for energy to all antigens of the test panel. Studies of lymphocyte responses to antigens before administration of transfer factor revealed that his cells did not respond to antigenic exposure with thymidine incorporation or MIF production (Fig. 4).

R. P. was the product of a normal pregnancy but his developmental milestones were delayed. Mucocutaneous candidiasis first appeared at age 2 yr and resisted therapy with topical nystatin and amphotericin B. Intravenous amphotericin at age 4 yr and clotrimazole at age 8 produced remissions, but the lesions recurred shortly after cessation of therapy. When admitted to the NIH at age 9 yr the patient had extensive candidiasis of the oral cavity, scalp, skin, and nails. Infection with *Herpes zoster* was present over the right hemithorax.

Routine laboratory work disclosed mild anemia due to iron deficiency. The leukocyte count and differential were normal; there was no lymphopenia. Endocrine gland function was normal. The stool did not contain trypsin activity. The chest X-ray showed multiple cysts in the right middle lobe and an esophageal study disclosed a stricture at the level of the carina.

Immunologic studies indicated a generalized defect of

the cellular immune system that was more severe than the other patients. There were no responses to the antigens of the skin test panel, and no contact hypersensitivity developed after application of the sensitizing dose of CDNB. His lymphocytes did not respond to stimulation with antigens with increased thymidine incorporation or MIF production. In contrast to the other subjects of this study, stimulation of his cells with phytohemagglutinin, concanavalin in A, or allogeneic cells did not result in increased thymidine incorporation (Table I). Humoral responses such as immunoglobulins, isohemagglutinins, and the acute and convalescent antivaricella C-F antibody titers were normal. Except for the normal lymphocyte count, the immunologic profile and lymph node histology of the patient were similar to the Nezelof syndrome (18).

*Delayed cutaneous hypersensitivity.* As described in the case histories, none of the patients had positive responses to the naturally encountered antigens, and four patients expressed no hypersensitivity after application of 2000 μg of CDNB. In the fifth patient (D. L.), the cutaneous response to the 100 μg challenge was equivocal, producing 1 cm of erythema but no induration or vesiculation.

In four patients, skin tests applied after injection of transfer factor evoked responses to candida, SK-SD, and mumps, reactivities possessed by the transfer factor donor. Reactivity to histoplasmin, another test that was positive in the donor, did not occur in any recipient. The reason for this is unknown, however, other studies of passive transfer of delayed hypersensitivity to histoplasmin with intact (19, 20) or frozen-thawed leukocytes (21) have indicated that reactivity to this antigen is difficult to transfer.

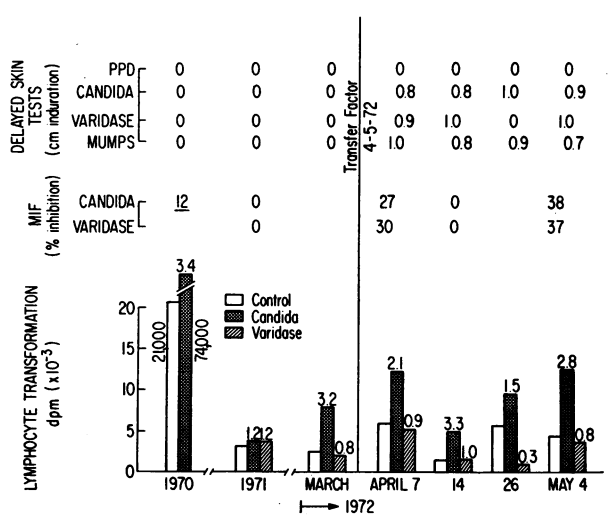


FIGURE 4 Effect of a single dose of transfer factor on in vivo and in vitro lymphocyte function in patient D. L.

TABLE I

*Antigen- and Mitogen-Induced Thymidine Incorporation by Lymphocytes from Control Subjects and Patient R. P.*

| Stimulant | Number of subjects | Mean stimulation ratio* and range: Control subjects | Mean stimulation ratio* and range: Patient R. P. |
|---|---|---|---|
| *Candida albicans* (5 μg/ml) | | | |
| Skin test negative | 7 | 1.3 (0.8–3.5) | 0.9 |
| Skin test positive | 10 | 20.8 (9.0–30.7) | |
| SK-SD (50/12.5 U/ml) | | | |
| Skin test negative | 5 | 2.6 (1.0–4.5) | 1.3 |
| Skin test positive | 7 | 47.7 (23.5–62.0) | |
| Phytohemagglutinin (1 μg/ml) | 20 | 436.0 (94–744) | 4.7 |
| Concanvalin A (20 μg/ml) | 7 | 100.0 (21–203) | 4.4 |
| Mixed leukocyte reaction with Phenotypically disparate, unrelated cells | 15 | 320.0 (18–450) | 1.1 |

* Stimulation ratio = mean dpm in stimulated cultures divided by mean dpm in identical cultures that contained no stimulant.

The fifth patient, R. P., had a more severe defect in cell-mediated immunity and did not develop any positive skin tests after two doses of transfer factor. In no case did a reactivity that was not possessed by the donor appear in a recipient. The results of serial skin tests in chronic recipients of transfer factor are summarized in Fig. 1–3. In these studies, the skin tests were applied and read just before the next injection of the transfer factor.

The duration of cutaneous responsiveness varied with different antigens and different recipients. After the first injection of transfer factor, the candida response in R. V. reverted to negative on the 5th day, but reactivity after subsequent injections persisted for over 2 months (Fig. 3). In contrast, the transferred candida response in J. A. C. persisted for approximately 60 days after the first injection of transfer factor. In general, reactivity to SK-SD persisted longer than reactivity to candida.

Induction of contact allergy to CDNB was attempted again with R. H., J. A. C., and R. V. while the recipients were responsive to other antigens through the action of transfer factor. The methods of sensitization and challenge were identical to those described above. In no recipient was sensitization achieved even though each subject was currently responsive to candida, SK-SD, and mumps.

*Lymphocyte transformation.* Antigen-induced lymphocyte thymidine incorporation was studied with cells cultured in autologous plasma, fetal calf serum, and in most cases, homologous plasma. The results were qualitatively similar in each experiment; there were no instances in which cells which were unresponsive in one medium became responsive when cultured in homologous or heterologous plasma or serum. The data in the figures and

Table I are from experiments conducted with 20% autologous plasma.

Skin test-negative control subjects frequently showed small increases in thymidine incorporation with candida or SK-SD. These were usually two- to threefold increments, but rarely fourfold increases were observed. In contrast, lymphocytes from skin test-positive control subjects responded to antigenic stimulation in vitro with 9- to 60-fold increments of thymidine incorporation, and even greater increments of thymidine incorporation occurred in mixed leukocyte cultures or mitogen stimulated cultures.

Lymphocytes from four patients (J. A. C., R. V., R. P., and D. L.) did not respond to in vitro antigenic stimulation by increased thymidine incorporation, but the lymphocyte transformation responses by R. H. were normal. Administration of transfer factor did not convert the thymidine incorporation responses by R. V. or D. L. even though the cutaneous responses were positive. With R. V., after the third injection of transfer factor on 24 September 1971 there was a marked increase in the rate of thymidine incorporation in both unstimulated and antigen-containing cultures. The reason for this change in base line activity was not identified.

After transfer factor, the thymidine incorporation by lymphocytes from J. A. C. was variable. In four of seven experiments her cells responded vigorously to SK-SD with stimulation ratios of 7.4–22 and one occasion, 5 February 1971, was there a significant response to candida. The lymphocyte transformation responses by R. H. were positive before transfer factor and there were no consistent changes in the magnitude of the responses or the dose-response relationship after the injection of transfer factor.

*MIF production.* Serial studies of antigen-induced MIF production are summarized in Figs. 1–4. Underlined numbers indicate that the inhibition of migration in culture fluids from antigen-stimulated cells did not differ significantly ($P > 0.05$) from control fluids. The studies with R. V. were compromised by leukopenia and adequate numbers of cells were not always available for all studies.

A consistent change in lymphocyte function in recipients of transfer factor was production of MIF, and in most cases, MIF production correlated with delayed skin test reactivity. The converse was observed with patient R. P. in whom transfer factor did not cause conversion of skin tests or in vitro MIF production.

The results of the immunologic studies are summarized in Table II. Conversion of negative skin tests to positive occurred in four patients and in each case was accompanied by MIF production by antigen-stimulated lymphocytes. In only one patient did the previously negative lymphocyte transformation reaction become positive and this was an inconstant finding.

## DISCUSSION

During recent years a variety of metabolic and immunologic abnormalities have been recognized in patients with infections due to *C. albicans*. Most frequently observed have been associations between candidiasis and functional abnormalities of the thymus-dependent or cellular immune system. Children with the DiGeorge or Nezelof syndromes of congenital absence of the thymus frequently have infections with *C. albicans*. In three instances, restoration of immune competence with thymus grafts was accompanied by clearing of candidiasis (22–24). Candidiasis has also been observed in subjects with thymoma (25–27), and patients with chronic mucocutaneous candidiasis often fail to express delayed cutaneous hypersensitivity to candida extracts (15, 28, 29).

From studies of cellular hypersensitivity in vivo it has been possible to segregate patients with chronic mucocutaneous candidiasis into three categories: patients with generalized defects in cell-mediated immunity such as the subjects of this study, patients in whom the cellular defects are limited to candida antigens, and patients in whom no cellular immune abnormalities are recognizable (15). In general, patients in the first category have had candidiasis since infancy or early childhood, suggesting a congenital defect in the cellular immune system. The age of onset in the other two groups is variable, and the basis of the underlying abnormalities of cellular immunity is poorly understood.

In vitro studies of lymphocytes from candidiasis patients in whom cellular immune defects are present have provided additional evidence for defective cellular immunity and have further illustrated the complexity of this syndrome. Although some patients possess serum factors which inhibit cellular immune functions (30, 31), most studies have established primary defects in lymphocyte function. Cells from some candidiasis patients fail to respond to stimulation with antigens, especially candida, with either increased thymidine incorporation or MIF production (15). Other patients have a different abnormality in which the replicative responses to antigens are normal, but MIF production is deficient (16, 32). A third aberrant pattern in patients with candidiasis was reported recently by Goldberg, Bluestone, Barnett, and Landau (33). Their patient had cutaneous anergy and normal MIF production, but her lymphocytes did not incorporate thymidine upon stimulation with candida.

The concept of treatment of chronic or recurrent infectious diseases by restoration of host defects is not new. It has long been an essential part of management of hypogammaglobulinemia and during recent years has been extended to disorders with isolated deficiencies of the

TABLE II

*Summary of Immunologic Responses by Candidiasis Patients before and after Transfer Factor*

| Patient | Before transfer factor | | | | After transfer factor | | | |
|---|---|---|---|---|---|---|---|---|
| | Skin tests | | Lymphocyte transformation | MIF production | Skin tests | | Lymphocyte transformation | MIF production |
| | Candida | CDNB | | | Candida | CDNB | | |
| R. H. | Neg* | Neg | Pos | Neg | Pos | Neg | Pos | Pos |
| J. C. | Neg | Neg | Neg | Neg | Pos | Neg | Intermittent pos | Pos |
| R. V. | Neg | Neg | Neg | Neg | Pos | Neg | Neg | Pos |
| D. L. | Neg | Equivocal pos | Neg | Neg | Pos | Not done | Neg | Pos |
| R. P. | Neg | Neg | Neg | Neg | Neg | Not done | Neg | Neg |

* Neg = negative result, pos = positive result.

thymus-dependent immune system and patients with combined immunologic deficiency syndromes. In a few cases, including a patient with chronic mucocutaneous candidiasis, bone marrow transplantation produced reconstitution of cellular immune function and therapeutic benefit (34). In other patients, similar reconstitutive efforts produced no benefit and in some cases were accompanied by severe graft vs. host reactions (35).

Lawrence has shown delayed cutaneous hypersensitivity can be transferred to normal subjects with negative skin tests by injection of extracts of leukocytes from skin test-reactive donors. This transfer factor is apparently not antigenic and does not cause graft vs. host reactions. Recently, transfer factor has been evaluated as a therapeutic adjunct in patients with the Wiskott-Aldrich syndrome, Swiss-type agammaglobulinemia, leprosy, and chronic mucocutaneous candidiasis (2–6). Assessment of therapeutic responses has been difficult for several reasons. The number of cases treated has been small and there is considerable heterogeneity among patients with the disorders. For example, patients with the Wiskott-Aldrich syndrome whose monocytes lacked a receptor for IgG became skin test-positive and derived clinical benefit from transfer factor, while those whose cells possessed this receptor did not (36). Although patients with lepromatous leprosy developed erythema and induration of lepromatous skin infiltrates after administration of transfer factor or viable lymphocytes from donors reactive to *Mycobacterium leprae,* there were no beneficial, or deleterious clinical effects (4).

In our study, transfer factor was evaluated as a therapeutic agent both alone and in association with intravenous amphotericin B. In the dosage schedule employed, transfer factor alone did not appear to be an effective treatment. These findings must be interpreted cautiously because the optimal dosage or treatment schedules, if any, are unknown. None the less, doses sufficient to convert negative delayed skin tests to positive, and which were accompanied by MIF production were not adequate to effect clearing of the mucocutaneous lesions.

On the other hand, our studies and reports from others suggest that remissions in chronic mucocutaneous candidiasis induced by anti-fungal therapy may be prolonged if cellular immunity is restored. In 1968, Buckley and coworkers reported an anergic patient with chronic candidiasis in whom treatment with both amphotericin B and bone marrow transplantation produced a remission which has persisted several years (34). Schulkind, Adler, Altemeier, and Ayoub (5), Pabst and Swanson (6), and Conway[8] have each treated a candidiasis patient with amphotericin B and transfer factor and observed sustained remissions. With the exception of oral candidiasis after antibiotic therapy in case R. H., both of our patients have been in remission for nearly 1 yr.

It is now established that optimal antibody synthesis by experimental animals requires interactions of two, and possibly three types of cells (37), and Asofsky, Cantor, and Tigilaar have presented evidence of cell cooperation in expression of a cell-mediated immune response, the graft vs. host reaction (38). Indirect evidence for multiple cell interactions in delayed cutaneous hypersensitivity has come from in vitro studies of lymphocytes from normal subjects and candidiasis patients. For example, Zoschke and Bach have used bromodeoxyuridine and light to selectively kill lymphocytes replicating in response to antigens (39), and Rocklin and Ratcliff have recently reported that cells that survive this treatment are still able to produce MIF when stimulated with the same antigen (40). While this finding may be explained by variations in the antigen dose required to initiate replication or by differences in doubling times, it could also occur if lymphokine-producing cells were distinct from transforming or replicating cells. Most anergic candidiasis patients fail to respond to antigenic stimulation with either thymidine incorporation or lymphokine production. These patients presumably have a defect either in differentiation of both antigen-responsive cell types or in the function of a common precursor cell.

[8] Conway, B. Personal communication.

Other patients have normal replicative responses, but do not respond to antigens with MIF production, and therefore would have a defect in "mediator-producing" cell line. The patient recently reported by Goldberg, Bluestone, Barnett, and Landau (33) would represent the other possible variant in which lymphokine production is preserved, but the replicating population is defective.

The multicellular model for delayed hypersensitivity permits consideration of possible mechanisms of action of transfer factor, including direct effects on either cell type, or on interactions or messages between cells necessary for expression of this immune response. The effects of transfer factor on antigen-induced replicative responses by lymphocytes have been variable. Lawrence (2) and Fireman, Boesman, Haddad, and Gitlin (41) have reported that lymphocytes from tuberculin-negative, normal subjects responded to in vitro stimulation with tuberculin after incubation with transfer factor from a tuberculin-positive donor. Schulkind et al. (5) have subsequently reported similar findings in a normal control and a patient with chronic candidiasis. The candidiasis patient was especially provocative because the data showed that incubation of lymphocytes in media containing transfer factor and antigen caused a shift in the dose-response curve. Peak thymidine incorporation responses in the absence of transfer factor occurred when 5 $\mu$l of candida antigen were added to the cultures. However, in cultures containing transfer factor from a candida-reactive donor, 5 $\mu$l of antigen did not increase thymidine incorporation. Enhanced thymidine incorporation in these cultures did occur when 10 $\mu$l of antigen was added and was even greater with 15 $\mu$l, the largest dose studied. Furthermore, a skin test-negative candidiasis patient developed a positive thymidine-incorporation response after injection of a leukocyte extract from a candida-responsive donor (8). His candida skin test remained negative.

However, other investigators have failed to observe antigen-induced replicative responses by lymphocytes from immunologically deficient recipients of transfer factor, even though the patients' delayed skin tests had become positive. The subjects of these studies had the Wiskott-Aldrich syndrome (3) lepromatous leprosy (4), and chronic candidiasis (6). Furthermore, although candidiasis patients R. V. and D. L. in our study became skin test-responsive after transfer factor, in vitro stimulation of their lymphocytes with antigen still did not increase thymidine incorporation. The responses of patient J. A. C. were inconstant, but frequently positive (Fig. 2). While the reasons for these diverse findings are unclear, they illustrate the heterogeneity of lymphocyte dysfunction in patients with candidiasis. Failure to regularly associate transfer factor-mediated delayed hypersensitivity with antigen-induced replicative responses suggests that transfer factor does not act primarily on antigen recognition by the replicating lymphocyte population. This proposition is supported by the fact that transfer of delayed hypersensitivity in both anergic and normal subjects occurs within 12–24 hr after administration of transfer factor, a time period that is incompatible with substantial mitotic expansion of a clone of antigen-responsive cells.

On the other hand, conversion of delayed cutaneous reactivity after administration of transfer factor has been associated with MIF production by cultured peripheral blood lymphocytes in patients with the Wiskott-Aldrich syndrome (3) and candidiasis (5, 42) including four of the subjects of this study. From in vitro studies, it is known that lymphokine production occurs before cell division and consequently is not dependent upon antigen-induced mitogenesis. It has also been established that production of these substances is antigen-specific, and does not occur in the absence of a previously sensitized lymphocyte population (43). In this regard, transfer factor could function by providing antigen-specific receptor sites to cells of indifferent specificity. Alternatively, if at least some lymphokine-producing cells possess antigen-specific receptor sites, transfer factor plus antigen may directly trigger these cells to produce MIF, a event that may result in nonspecific recruitment of additional cells into lymphokine production. Production of MIF without conversion of antigen-induced replicative responses in most anergic recipients of transfer factor detracts somewhat from this explanation since it supposes that the antigen-specific receptors of the replicating and lymphokine-producing cells are unique, have different degrees of sensitivity for stimulation, or that the functional deficiencies in these patients' lymphocytes could not be understood as single defects.

A third possible mechanism of transfer factor is that it replaces an antigen-dependent interaction or message between lymphokine-producing cells and some antigen-response cell pool. Clearly, since both transfer factor and antigen are required for development of positive skin tests, the mechanism must be different from that leading to nonantigen-dependent "delayed hypersensitivity-like" reactions after intradermal injections of the nonspecific phytomitogens concanavalin A (44) and phytohemagglutinin (45). This mechanism would be consistent with induction of delayed hypersensitivity both in normal subjects and in patients with functional defects either of antigen recognition or lymphokine production. It might also explain the apparent paradox in lymphocyte functions in vitro after transfer factor administration.

The apparent failure of transfer factor to induce development of delayed allergy in patients with profound

defects in cellular immune functions such as R. P. in our study is consistant with any of the proposed mechanisms. One would predict that only certain defects could be abrogated by transfer factor and that more severe or fundamental deficiencies would be less amenable to restitution. It is apparent that further investigations of patients with immunological deficiencies will be useful for understanding the biologic basis of transfer of delayed hypersensitivity by soluble agents.

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