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Immunotherapy with Transfer Factor in Disseminated Coccidioidal Osteomyelitis and Arthritis

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TRANSFER FACTOR (TF), first prepared by Lawrence from human lymphocytes,¹ is a chemical moiety with a molecular weight of less than 10,000. It is dialyzable and unaffected by ribonuclease, deoxyribonuclease and trypsin.²

TF has been used in treating diseases in which cellular immunity is deficient. A few patients with congenital immune deficiency states such as Wiskott-Aldrich syndrome, severe combined immunodeficiency disease and dysgammaglobulinemia, have improved following therapy with TF.³ TF has been administered to patients with infectious diseases in which cellular immunity is deficient. Examples of such diseases are candidiasis,⁴⁻⁶ lepromatous leprosy⁷ and disseminated tuberculosis.⁸ It also has been used in attempts to restore cellular immunity in diseases with acquired cellular immune deficiency states such as sarcoid and carcinoma.^{9,10}

Coccidioidomycosis is one of the fungal diseases in which the primary site usually is minimally symptomatic or asymptomatic pulmonary infection without progression of disease. Healing of this primary process is associated with the development of cellular immunity as manifested by a delayed hypersensitivity reaction to intradermal testing with coccidioidin antigen. Failure to mani-

fest delayed hypersensitivity in a patient with disseminated disease has been associated with a prolonged clinical course despite chemotherapy. The following case is that of a patient who had disseminated coccidioidomycosis with osteomyelitis who was not responding to amphotericin B therapy. He had remained anergic for several months, and then was treated with transfer factor.

Report of a Case

A 24-year-old man of Spanish-American extraction was admitted to hospital after four months of progressive swelling involving multiple joints. In the week before admission chills and fever and drainage from the right ankle had developed. The patient denied intravenous use of drugs, venereal disease, trauma or antecedent arthritis. He had not traveled outside the Los Angeles-Orange County area for ten years.

The temperature on admission was 38.8°C (102°F). The pulse rate was 132 and regular. Ankles, knees, elbows, and the right wrist had tender, swollen, fluctuant and erythematous areas of varying severity. Laboratory data on admission to hospital included hemoglobin of 9.8 grams per 100 ml, and 13,500 leukocytes per cu mm with 78 percent polymorphonuclear cells, 18 percent lymphocytes, and 4 percent eosinophils. The erythrocyte sedimentation rate was 61 mm in one hour (Westergren). Blood urea nitrogen was 11 mg and serum creatinine 0.7 mg per ml. An x-ray film of the chest was normal. Radiographs of all the involved bony areas, as well as both femurs and ischium, were interpreted as showing diffuse osteomyelitis with bone destruction. *Coccidioides immitis* was cultured from aspirates or drainage from the right hand, left elbow, both knees, both ankles, the left foot and from bone marrow. The intracutaneous test to coccidioidin 1:100 was negative (Table 1) and the complement fixation antibody titer against coccidioidin was positive at 1:2048.

Therapy consisted of administration of amphotericin B, surgical excision and drainage, and appropriate antibiotics for several bacterial superinfections. After a month of such therapy, and 1.5 grams of amphotericin B administered intravenously, the patient's serum creatinine was 1.3 mg per 100 ml and creatinine clearance was 50 ml per minute. At the fifth month the creatinine clearance had declined to 28 ml per minute. The patient was febrile. Body weight had dropped to 80 pounds from a normal of 150 pounds. Cul-

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TABLE 1.—Results of Intracutaneous Tests for Delayed Hypersensitivity before and after Administration of Transfer Factor

Skin Test	DATE								
	7/70	11/70	2/71	1/71	3/71	4/71	6/71	3/72	7/72
TF Administration			×			×			
Coccidioidin (Cutter) 1:100	0	0		0	0		E=20 mm I=5 mm	E=? I=19 mm	E=60 mm I=19 mm
PPD 5 US units (Connaught)	0	0		0	0	0
Histoplasmin (Parke-Davis) Standardized	0	0		0	0	0
Mumps (Eli Lilly) 2 CF units	0		0	I=15 mm	I=10 mm
Trichophyton 1:30 (Hollister-Stier)	0		0	0
Dermatophytin 1:100 (Hollister-Stier)	0		0

× = Date administered.
 0 = Negative result (No induration or erythema).
 .. = Not tested.

E = Erythema.
 I = Induration.

tures positive for *C. immitis* were still obtained from draining lesions.

The complement fixation titer decreased one dilution to 1:1024, and skin tests to both 1:100 and 1:10 concentrations of coccidioidin were still negative.

The decision was made to employ transfer factor immunotherapy in an attempt to improve cellular immunity to *C. immitis* because of the deteriorating condition of the patient and the evidence of renal toxicity from amphotericin. For this purpose an extract* obtained from 1 unit of blood donated by a coccidioidin hypersensitive donor was used. No evidence was provided for activity of this material. Following this administration of TF there was no conversion of the coccidioidin skin tests. However, the patient's clinical status stabilized and no new drainage area appeared. A second dose of TF was administered two months after the first. This material was prepared at our laboratories with 500 ml of blood from another donor. (See Methods.)

When skin tests were applied six weeks after the second administration of TF, a positive coccidioidin skin test was obtained for the first time (Table 1). The patient was discharged to a convalescent home and for the next six months continued to receive amphotericin but at a reduced schedule, as suggested by Winn when renal toxicity is a factor.¹² The maintenance dose of 40 mg of amphotericin B administered intravenously every third week was interrupted for six weeks,

*1.8 ml was provided by Dr. Peter Baram, University of Chicago.

followed by a mild relapse with fever, pain and tenderness in the right elbow. *C. immitis* was cultured from an aspirate of the involved site. The lesion gradually healed after weekly administration of amphotericin therapy was resumed.

Three months later, 21 months after his original admission, the patient had received a total of 6.335 grams of amphotericin B. Creatinine clearance values were between 18 and 22 ml per minute. The coccidioidin skin test remained positive and the complement fixation titer had decreased to 1:256. The patient felt well, and there was radiographic evidence of healing of the bone lesions. At this point amphotericin was discontinued.

In the 16 months of follow-up, the patient remained free of symptoms except for a small draining lesion on the right wrist which cultured *C. immitis*, but which cleared spontaneously. He regained most of his lost weight and felt well. Some residual joint deformities and limitation of motion remained.

Methods

Transfer Factor Preparation

A coccidioidin-positive donor was located and a unit of blood was obtained. The red cells were removed from the whole blood by sedimentation for several hours at 4°C (39.2°F). The buffy coat was removed, and the cells washed several times in 15 millimols of sodium chloride. Then they were suspended in a small quantity of distilled water, frozen and thawed a total of five times and dialyzed against 50 ml of distilled water

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TABLE 2.—DNA Synthesis† and LT Production from Purified Peripheral Blood Lymphocytes Cultured with Antigens and Mitogens *in vitro* before and after Treatment with TF.

Stimulants	Before Admin. of TF (Skin Test Negative)	After 1st Dose of TF (Skin Test Negative)	After 2nd Dose of TF* (Skin Test Positive)
PHA 30 µg	37,577 CPM ± 12%	32,010	50,357 ± 10%
PWM .01 ml	17,481 CPM ± 10%	17,647	22,781 ± 12%
Coccidioidin 0.01 ml . . .	1,201 CPM ± 20%	872	1,372 ± 15%
Coccidioidin 0.05 ml . . .	876 CPM ± 30%	998	4,577 ± 8%
Coccidioidin 0.1 ml . . .	1,205 CPM ± 23%	1,510	4,763 ± 7%
Histoplasmin 10 µg	987 CPM ± 20%	1,213	897 ± 30%
None	1,105 CPM ± 20%	939	1,041 ± 30%
LT production	0	0	+

*Prepared from a different donor than the first dose of TF.
†Incorporation of H³ Thymidine.

DNA = Desoxyribonucleic acid
LT = Lymphotoxin

H³ = Tritium
PHA = Phytohemagglutinin

PWM = Pokeweed mitogen
CPM = Counts per minute, H³ thymidine incorporation

for 24 hours. The dialysate was shell frozen, lyophilized to dryness and reconstituted to a total volume of 2 ml with distilled water. The salt concentration in the TF sample was adjusted to physiologic levels. All procedures were performed under aseptic conditions.

Immunological Studies

Screening immunologic tests were done. These included intracutaneous tests, serum complement fixation tests and tests of the response of the patient's lymphocytes to various stimuli. The patient's lymphocytes were tested *in vitro*, before and after injection of transfer factor, for the capacity to synthesize DNA and produce lymphotoxin (Kramer and Granger¹¹).

Results of Immunological Studies

Results of immunological studies are summarized in Tables 1 and 2. *In vitro*, the patient's lymphocytes responded to phytohemagglutinin (PHA) and pokeweed mitogen (PWM), but did not respond to three levels of coccidioidin, and produced no lymphotoxin. One dose of TF produced no change in the skin test or in lymphocyte reactivity. After a second dose of TF, which was prepared in our laboratory from the blood of another donor, the skin test converted, coccidioidin-induced lymphocyte DNA synthesis increased fourfold and lymphotoxin was produced. These apparent improvements in immunologic responses occurred during the same period that amphotericin was being administered and the patient was beginning to improve clinically.

Discussion

Morbidity and mortality rates associated with disseminated coccidioidomycosis have been high, with an overall mortality of 50 percent re-

ported before amphotericin B became available.¹² With amphotericin therapy, the outlook is better.¹³⁻¹⁷ In one review of 42 patients with disseminated coccidioidomycosis who were treated with intravenous amphotericin B, 82 percent had rapid and significant therapeutic responses. The dose range in 30 of these successfully treated patients was from 0.9 gram to 6.92 grams, with an average total dose of 2.47 grams.¹⁸ The patient in the present case received 6.33 grams.

About 20 percent of disseminated coccidioidomycosis involves bone.¹⁵ In one series 43 patients had coccidioidal involvement of the bones. Of these patients 21 were treated before 1957 and therefore did not receive amphotericin B. Eight of this group died, three had persistent drainage and ten were apparently cured. Of the 22 patients treated with amphotericin B after 1957, 19 were completely cured, one had persistent drainage and two died.¹⁵ It is therefore apparent that therapeutic failures to treatment with amphotericin B still occur, and interference with normal renal function is an ever present danger.

Dissemination may be attributable to the virulence of the pathogenic organism, to the size of the infecting dose or to impaired host resistance. That the patient studied in this report is of Spanish-American descent may have accounted for lower resistance, since the incidence of dissemination is greater in darker skinned people for unknown reasons.^{18,19}

Once dissemination occurs it is evident that the usual defense mechanisms, in particular the immunological ones, may be overwhelmed. Whether the patient in this case had a pre-existing defect in his immune defenses which facilitated dissemination is not known. Humoral antibodies of the precipitin and complement fixation types are produced in large amounts, while specifically sensi-

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tized thymus-dependent lymphocytes are difficult to detect. These thymus lymphocytes presumably help destroy the invading organism while the antibodies may interfere with this activity by blocking access of the foreign antigens to the lymphocyte receptors.

Disseminated coccidioidomycosis, especially in the meningeal form, is well known for protracted courses of exacerbations and remissions. The disease can remain quiescent for long periods and engender false hope of a cure. Evaluation of any other treatment is therefore difficult. In the case of transfer factor immunotherapy, the problem is compounded by several unknown factors. To list only a few, there is the lack of knowledge of chemical structure, standardization of assay methods and specificity of action. In its favor, TF is apparently non-toxic and non-antigenic. It is hypothesized that in some unknown way TF from a coccidioidin skin test-positive donor stimulates the recipient's thymus lymphocytes to proliferate and attack *C. immitis* antigens either by direct contact or by the secretion of lymphokines such as lymphotoxin. Therefore, co-treatment with a fungicidal agent and TF may serve complementary aims. It could lessen the antigen load by destroying the growing organisms and also enhance cellular immunity by stimulating the production of specific thymus lymphocytes.

Progression of active lesions of coccidioidomycosis despite doses of amphotericin in the range of renal toxicity appears to be logical indication for immunological intervention in the form of transfer factor administration. A single trial, although a start, cannot confirm the efficacy of such an approach. Controlled studies are needed, as well as a method of standardizing the preparation and dose schedule of transfer factor before a definitive judgment can be made.

Summary

A patient with disseminated coccidioidomycosis involving the bones was moribund despite doses of amphotericin B in the renal toxicity range. He

remained anergic to coccidioidin. After the second administration of Lawrence's transfer factor (TF) in an attempt to reconstitute the patient's cellular sensitivity to coccidioidal antigen, the patient improved clinically. *In vivo* and *in vitro* tests for cellular immunity to *C. immitis* became positive.

It is not known whether these favorable changes were the result of TF immunotherapy or coincident with it. The effectiveness of such therapy will remain uncertain without controlled trials in patients with disseminated fungal disease and standardized TF assays.

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