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# TREATMENT OF CHRONIC MUCOCUTANEOUS MONILIASIS BY IMMUNOLOGIC RECONSTITUTION

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## SUMMARY

The immunological defect in a patient with chronic mucocutaneous moniliasis was characterized. While his Candida skin test was negative. exposure of his lymphocytes to candida extracts *in vitro* produced an increase in thymidine incorporation. Supernatants from cultures of antigen-stimulated lymphocytes did not contain macrophage migration-inhibition factor (MIF) activity.

Restoration of the immune system with transfusions of immuno-competent allogeneic lymphocytes was accompanied by conversion of the Candida skin test to positive, and MIF production by his lymphocytes. During the period that his immune system remained intact, there was marked clearing of the moniliasis. Eight months following the transfusions, the moniliasis recurred and when restudied, the patient again had negative skin tests and insignificant MIF production.

These observations demonstrate the importance of mediators in the expression of delayed hypersensitivity and provide evidence of a role of cellular immunity in resistance to certain chronic fungal infections.

## INTRODUCTION

The role of immunologic factors in resistance to infection with *Candida albicans* is poorly understood, however, several observations have indicated the importance of the cellular immune system. Infants with congenital absence or dysplasia of the thymus have a high incidence of moniliasis (Hermans, Ulrich & Markowitz, 1969), and in one instance, restoration of immune competence with a thymus transplant, was accompanied by clearing of the monilia infection (Cleveland *et al.*, 1968).

Recently it has been shown that lymphocytes from animals or human subjects with delayed hypersensitivity respond to antigenic stimulation *in vitro* by replicating (Ling, 1968) and producing substances with diverse biological activities such as chemotaxis (Ward, Remold & David 1969), cytotoxicity (Granger & Williams, 1968), inhibition of migration

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of macrophages (David, 1968) and non-specific recruitment of lymphocytes into DNA synthesis (Maini *et al.*, 1968; Kirkpatrick *et al.*, 1970). These substances are presumed to be 'mediators' involved in the pathogenesis of lesions of cellular hypersensitivity.

This report describes a patient with chronic mucocutaneous moniliasis and hypoparathyroidism in whom a defect in the cellular immune system was characterized. Because amphotericin-B induced remissions in these patients are rarely sustained, it was elected to attempt immunologic reconstitution. Transfusion of immuno-competent lymphocytes temporarily corrected the defect and was accompanied by marked clearing of the moniliasis.

## CASE REPORT

The patient, a 17-year-old Caucasian male, was admitted to the National Institute of Allergy and Infectious Diseases in May 1969, for investigation of chronic mucocutaneous moniliasis. He developed severe 'diaper rash' at 6 months. *Candida albicans* was cultured from the eruption. The skin lesions progressed and by 18 months of age involved the scalp, face, ears, neck, back and distal extremities, as well as the nails and buccal cavity. At age 7 years the serum calcium and phosphorus levels were 10·5 and 4·5 mg per 100 ml respectively. At a routine examination 5 years later, the serum calcium and phosphorus concentrations were 6·5 and 9·4 mg per 100 ml although the patient had no symptoms of hypocalcemia. He was referred to the National Heart Institute where the diagnosis of primary hypoparathyroidism was established. Although there have been periods of drug resistance, in general the response to treatment with calcium gluconate, dihydrotachysterol or 25-hydroxycholecalciferol has been satisfactory.

Severe dysplasia of the dental enamel led to complete extractions in 1964. The extent and severity of the moniliasis have varied through the years, but the general course had been one of progression. There had been no sustained response to nystatin, gentian violet or topical amphotericin-B. With the exception of numerous hospitalizations for evaluation and treatment of moniliasis and an episode of pneumococcal septicemia at age 18 months, the past history was unremarkable. The patient had no unusual incidence of viral infections, and there were no known allergies.

Upon admission to the National Institute of Allergy and Infectious Diseases, the general physical examination revealed numerous erythematous, firm, elevated, crusting lesions over the scalp, neck (Fig. la), back, groin, and distal extremities (Figs 2a, 3a, 4a), and extensive destruction of the nails (Fig. 5). The external auditory canals were scaly and contained serous exudates. Both tympanic membranes were thickened and the right membrane had a large perforation. The mouth was edentulous and many large patches of thrush were present over the membranes of the tongue and buccal cavity. Several 1–2 cm firm, non-tender lymph nodes were felt in the cervical and supraclavicular areas, and the spleen tip was 2–3 cm below the left costal margin. Chvostek's sign was positive.

No other members of the family were known to have endocrinopathies immunological abnormalities or moniliasis, and there was no history of consanquinity.

Routine laboratory studies such as the peripheral blood count, urinalysis, blood urea nitrogen, creatinine, fasting and post-prandial glucose, electrolytes and liver function tests were normal. The serum calcium varied between 7·2 and 8·8 and the phosphorus between 5·0 and 5·9 mg per 100 ml. The serum iron was 65 $\mu$g per 100 ml. Studies of endocrine

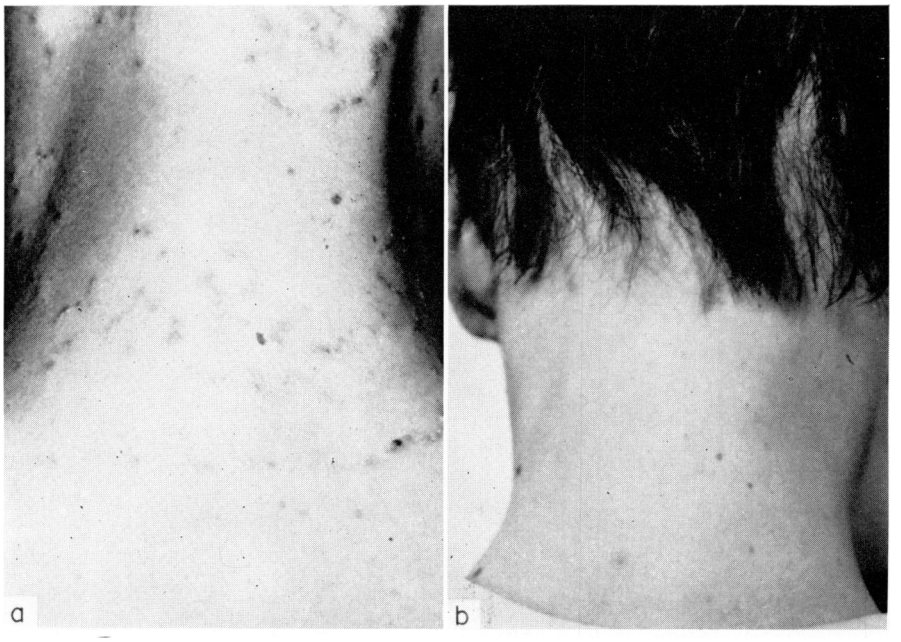


FIG. 1. (a) Cutaneous moniliasis involving the skin of the neck (May, 1969). (b) Marked clearing of the lesions occurred three weeks following the lymphocyte transfusions. There was also marked improvement of the alopecia.

gland and gastrointestinal functions are summarized in Table 1. Cultures of the buccal cavity, skin, scalp, and nails yielded *Candida albicans*, Group A, but there was no growth from the urine, blood or bone marrow.

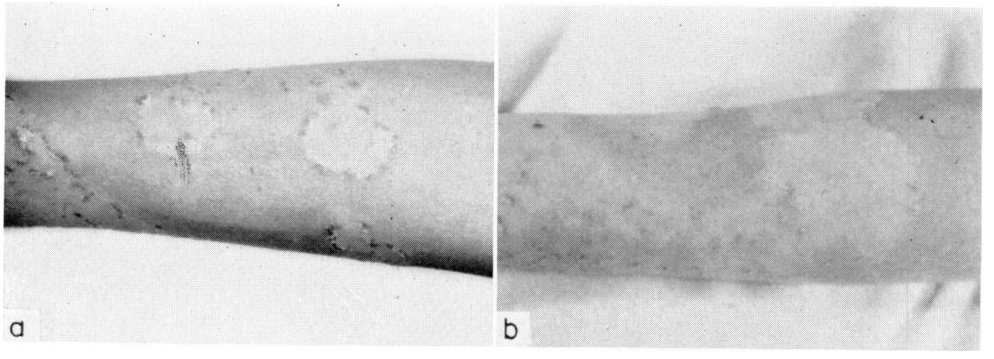


FIG. 2. (a) Cutaneous moniliasis of the skin of the forearm (May, 1969). (b) Same area three weeks after leucocyte transfusions. There was marked loss of the erythematous plaques with temporary depigmentation of the healed areas.

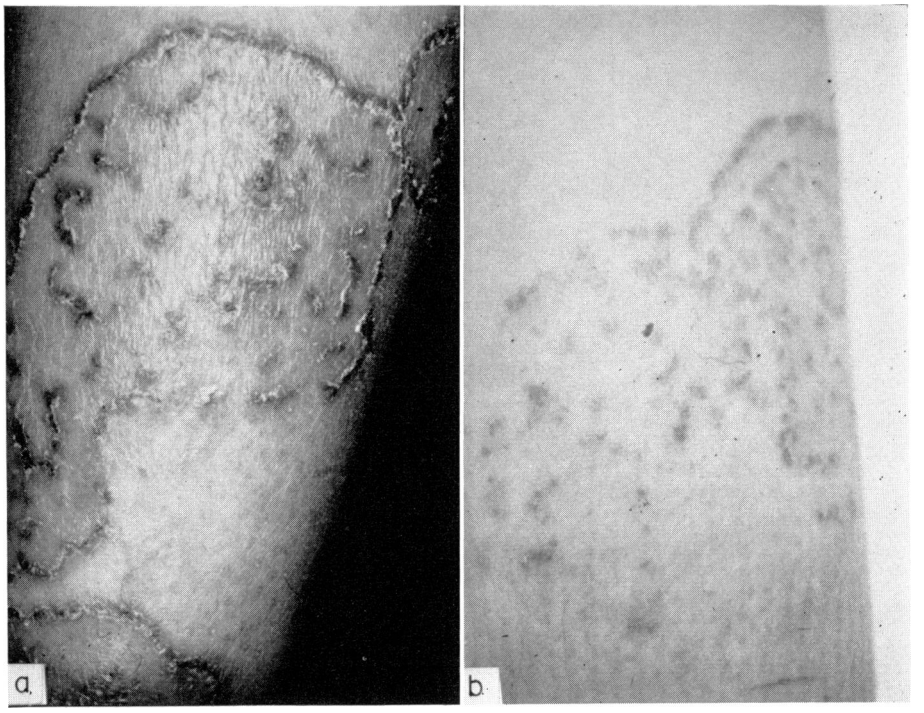


FIG. 3. (a) Moniliasis involving skin of the calf (May, 1969). (b) Three weeks after the leucocyte transfusions there was marked improvement in the cutaneous lesions. Only soft erythema remains.

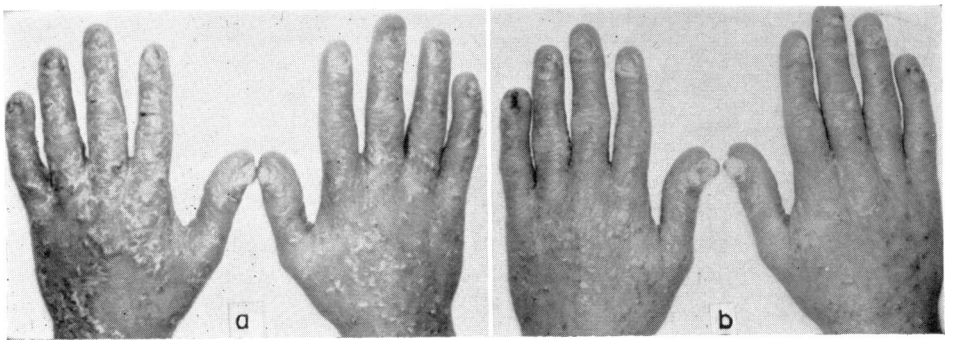


FIG. 4. (a) Moniliasis of the hands and nails (May, 1969). (b) Partial clearing of the cutaneous lesions 5 weeks following the leucocyte transfusions. There was essentially no change in the nails.

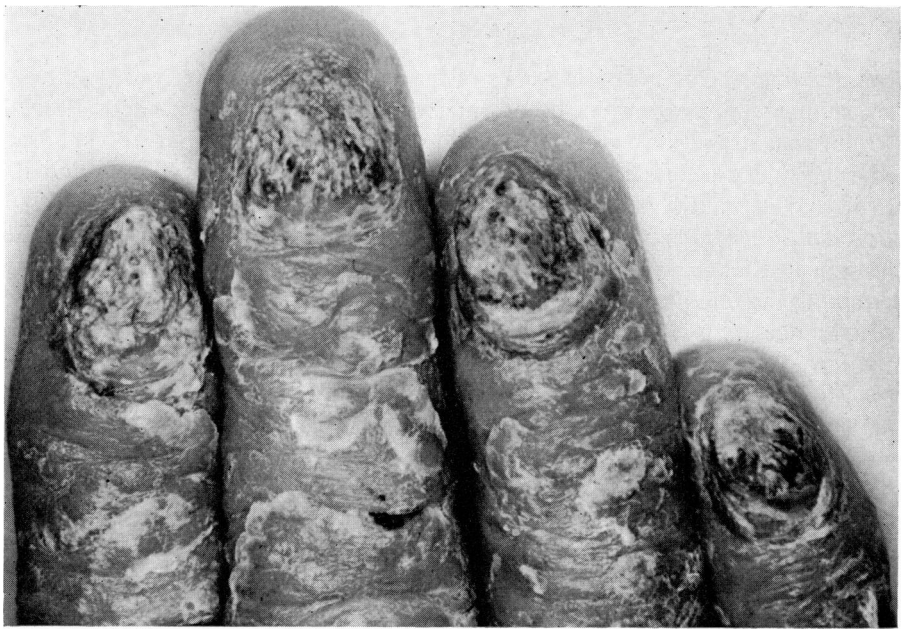

FIG. 5. (a) Chronic moniliasis involving the nails of skin of fingers.

TABLE 1. Endocrinological and gastrointestinal function studies

| | | |
|---|---|---|
| Serum calcium | | 7·30–8·80 mg/100 ml |
| Serum phosphorus | | 5·0–5·9 mg/100 ml |
| Parathormone test | Baseline | After 1500 units PTH |
| Serum Ca (mg/100 ml) | 6·30–8·40 | 12·8 |
| Serum P (mg/100 ml) | 9·0 –9·6 | 4·9 |
| ACTH Test | Baseline | Max. excretion after stimulation |
| 17-OH Steroids (mg/24 hr urine) | 5·6 | 21·8 |
| 17-Ketogenic Steroids (mg/25 hr urine) | 4·3 | 4·3 |
| PBI ($\mu$g per 100 ml) | 5·6 (Normal 4·0–8·0) | |
| $T_4$ (column) ($\mu$g per 100 ml) | 5·0 (Normal 3·1–6·7) | |
| $T_3$ (per cent) | 32·5 (Normal 25–35) | |
| Serum Carotene (units) 142 (normal 100–300) | | |
| D-xylose-1·8 g excreted in 5 hr after 5 g oral dose | | |
| Gastric acidity–Positive by Diagnex blue test | | |

## METHODS AND MATERIALS

*Intradermal skin tests.* Cutaneous responses of the patient and his father were studied with a panel of commercial antigens including intermediate and second strength PPD, histoplasmin, mumps, streptokinase-streptodornase (SK-SD, Varidase, Lederle Laboratories, Pearl River, New York) diluted to contain 400 units and 100 units or 40 units and 10 units per 0·1 ml, and dermatophytin (Hollister-Stier Laboratories, Downers Grove, Ill.) 1:100. An additional antigenic extract was prepared by sonically disrupting *C. albicans* isolated from the patient (Kirkpatrick, Chandler and Schimke, 1970). This material was standardized by determining the minimal dose producing 0·5 cm of induration at 24 or 48 hr following intradermal inoculation into healthy subjects known to have delayed allergy to *C. albicans*. Repeated intradermal tests applied to subjects with negative skin tests, demonstrated that the test antigen was not sensitizing in the dosages employed.

All antigens were injected in a volume of 0·1 ml and the cutaneous sites were examined at 15 min for immediate wheal and flare responses, 6–8 hr for Arthus-like reactions and 24 and 48 hr for delayed hypersensitivity. Delayed reactions with 0·5 cm of induration were considered positive.

Two thousand micrograms of 1-chloro 2,4-dinitrobenzene (CDNB) in acetone were applied to the medial aspect of the upper arm to induce contact allergy. Two weeks later the subjects were challenged by application of 50 $\mu$g and 100 $\mu$g of the substance of the flexor surface of the forearm.

*In vitro studies of lymphocyte functions.* The technique for measuring antigen-induced thymidine incorporation in short duration lymphocyte cultures differed slightly from that described previously (Newberry *et al.*, 1968). The tubes contained $2{\cdot}0 \times 10^6$ lymphocytes in a volume of 2·0 ml of Eagle's minimal essential medium with 15% autologous or homologous plasma, and the cells were exposed to tritiated thymidine during the final four hours of culture.

Macrophage migration inhibition factor (MIF) production in antigen-stimulated and control lymphocyte cultures was measured by methods similar to those described by Thor *et al.* (1968) and Rocklin, Meyers & David (1970). Ten micrograms of the soluble candida sonicate (Kirkpatrick, Chandler & Schimke, 1970) were added to the antigen-stimulated cultures at the beginning of the culture period and after each medium change. The media were collected daily for three days and pooled supernatants were concentrated five-fold by ultrafiltration. Control fluids containing tissue culture media from unstimulated cells or tissue culture media containing no cells, but the same amount of antigen were also prepared and concentrated in the same manner. The supernatants were then added to three Sykes-Moore chambers (Bellco Glass Co., Vineland, N.J.), each containing two capillary tubes filled with guinea-pig peritoneal macrophages. The areas of migration were measured at 16 hr and MIF activity was calculated by the method of Thor *et al.* (1968). Reduction of the area of migration by 20% or more was significant ($P<0{\cdot}02$).

Mixed lymphocyte cultures were performed using the 'one-way' method of Bach & Voynow (1966). The cells were labelled with tritiated thymidine during the last 4 hr, and the cultures were harvested on the seventh day.

*Serological studies.* Candida agglutinins in serum and parotid fluid were measured as described elsewhere (Kirkpatrick *et al.*, 1970) and precipitins were detected by double diffusion in agar gel (Ouchterlony, 1962).

The concentration of IgG, IgA and IgM in the serum was determined by radial diffusion (Mancini, Carbonara & Heremans, 1965) using commercial antibody containing agar gels (Hyland Laboratories, Los Angeles, Calif.). Rheumatoid factor, anti-nuclear antibodies, antithyroglobulin, and the lupus erythematosus factor were assayed by standard laboratory methods. Antibodies against gastric parietal cells, thyroid and adrenal tissues were measured in the laboratory of Dr Robert Blizzard.

*Phagocytosis and metabolic properties of leucocytes.* The capacity of the patient's and normal leucocytes to ingest and kill micro-organisms was studied with *S. albus*, and *C. albicans* (Mickenberg, Root & Wolff, 1970). Oxidative reactions of leucocytes subsequent to phagocytosis were studied in phagocytic mixtures as previously described (Mickenberg *et al.*, 1970).

*Histology of inflammatory exudates.* Migration of leucocytes into an area of cutaneous abrasion was studied as described by Rebuck & Crowley (1955).

*Leucocyte and erythrocyte typing.* Lymphocytes from the patient and members of his family were tested for HL-A antigens by the microcytotoxicity method of Mittal *et al.* (1968), and the HL-A genotypes were determined. Donor and recipient sera were also screened for preformed cytotoxic antibodies by the same technique.

Erythrocyte phenotypes were determined in the Clinical Center Blood Bank by standard methods.

*Leucocyte transfusions.* Leucocytes were collected from the patient's father using the NCI-IBM continuous flow blood cell separator (Buchner *et al.*, 1969). Four leucophoresis were performed in 5 days during which $92{\cdot}2 \times 10^9$ leucocytes containing $63{\cdot}6 \times 10^9$ lymphocytes were collected. The cells were collected in plastic bags (Transfer Pack, Fenwal Laboratories, Norton Grove, Ill.), containing acid-citrate-dextrose (ACD, NIH formula A) and were transfused into the recipient within 1 hr of collection.

## RESULTS

*Cutaneous hypersensitivity.* The patient developed no immediate or delayed cutaneous reactions to intermediate or second strength tuberculin, histoplasmin, streptokinase-streptodornase or Hollister-Stier dermatophytin, but 0·6 cm of erythema and soft induration appeared at the site of the mumps skin test (Table 2). Fifteen minutes after injection of 1·0 $\mu$g of the Candida sonicate, a 2·0 cm pruritic wheal and flare response occurred which subsided in 30 min. At 6 hr the injection site became somewhat indurated, tender and erythematous, but never haemorrhagic or necrotic. This response also subsided, and at 24 hr there was no residual erythema or induration. Subsequent studies with the same antigen in doses containing 0·1 to 100 $\mu$g of protein also failed to produce a delayed cutaneous response.

No vesiculation occurred at the site of application of the sensitizing dose of CDNB, and topical challenges with 50 and 100 $\mu$g 14 and 38 days later were negative.

The patient's father developed significant reactions to mumps, SK-SD and *C. albicans* and sensitization with 2000 $\mu$g of CDNB produced strong contact allergy.

From Table 2 it is apparent that passive transfer of delayed cutaneous responses to SK-SD, *C. albicans* and CDNB accompanied the leucocyte transfusions. Cutaneous responses to CDNB were still present at 14 days, but at 37 days only erythema was observed and subsequent tests were entirely negative. The cutaneous responses to SK-SD and

*C. albicans* remained positive for 190 days, but both had reverted to negative when he was re-studied at 315 days. Interpretation of the mumps skin test prior to transfusion was difficult because of the absence of definite induration. However, following the leucocyte transfusions well demarcated induration was found. This response also reverted to the previous soft induration at 315 days.

*Lymphocyte transformation. In vitro* stimulation of the patient's lymphocytes with the candida extract produced an increase in thymidine incorporation (Table 3). Peak responses occurred in cultures stimulated with 5·0 μg of antigen protein per ml, but other doses also

TABLE 2. Delayed cutaneous hypersensitivity response of the patient and his father before and after leucocyte transfusions

| Antigen | Recipient Pre-transfusion | Donor (Father) | Recipient Post-transfusion (days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 7 | 14 | 24 | 37 | 149 | 190 | 315 |
| Mumps | 0·6* | 1·8 | 0·5 | 0·3 | 1·5 | 1·5 | 0·9 | 0·7 | 0·7 | 0·6 |
| *C. albicans* | | | | | | | | | | |
| Hollister-Stier 1:100 | 0 | 0 | 0 | 0 | 0·5 | 0 | 0·7 | 0·9 | 0·5 | 0 |
| Autologous *C. albicans* | | | | | | | | | | |
| 100 μg | 0 | — | — | — | — | — | — | — | — | 0 |
| 10 μg | 0 | 2·0 | 0·2 | 0·9 | 1·0 | 1·2 | 1·7 | 0·9 | 1·1 | 0 |
| 1 μg | 0 | 0·4 | 0 | 0 | 0 | 0 | 0·5 | 0·7 | 1·0 | 0 |
| SK-SD | | | | | | | | | | |
| 4000/1000 units | 0 | — | — | — | 1·2 | 1·4 | 1·0 | 0·9 | 0·8 | 0 |
| 400/100 units | 0 | 9·0 | 0·5 | 0·7 | 0 | 1·9 | 0·5 | — | — | 0 |
| CDNB | | | | | | | | | | |
| 100 μg | 0 | + | + | | + | | eryth 0 | | 0 | |
| PPD | 0 | 0 | 0 | | — | | 0 | | 0 | |
| Histoplasmin | 0 | 0 | 0 | | — | | 0 | | 0 | |

* cm induration at 24 hr.

provided some stimulation. Although the amplitude of the thymidine response was somewhat lower than control subjects, peak responses with the patient's cells occurred at the same dose. Mumps skin test antigen that had been dialyzed free of merthiolate, and phytohaemagglutinin-M also stimulated thymidine incorporation by the patient's lymphocytes. No significant changes in the thymidine response were noted when the patient's cells were cultured in homologous rather than autologous plasma, and the patient's plasma had no effect on the responses of cells from skin test positive donors. Although not shown on the table, subjects with negative skin tests had a two-fold or less increase in thymidine incorporation when their lymphocytes were stimulated with *C. albicans in vitro*.

Following the leucocyte transfusions, neither the magnitude of the response nor the antigen dose producing maximal stimulation were changed.

TABLE 3. Incorporation of tritiated thymidine by antigen and mitogen stimulated lymphocyte cultures from the patient, his father and healthy skin test-positive subjects

| Antigen | Mean disintegrations per min | | | | |
|---|---|---|---|---|---|
| | Patient | Father | Skin test positive normals | | |
| | | | 1 | 2 | 3 |
| *C. albicans* sonicate | | | | | |
| 0 (control) | 1120 | 840 | 2040 | 4220 | 4000 |
| 50 μg/ml | 780 | 865 | 1355 | 3570 | 1800 |
| 5 μg/ml | 6330 | 15255 | 17640 | 27960 | 53700 |
| 0·5 μg/ml | 5700 | 3240 | 4040 | 7620 | 7405 |
| 0·1 μg/ml | 1030 | 239 | 1740 | 2700 | 1710 |
| 0·05 μg/ml | 825 | — | 1200 | — | — |
| Mumps | | | | | |
| 0·1 ml | 9490 | — | 10260 | — | — |
| PHA-M | | | | | |
| 0·1 ml | 310770 | 507000 | 160500 | 99320 | 246000 |

*Macrophage migration inhibition factor.* Prior to the leucocyte transfusions, concentrated supernatants from the patient's antigen-stimulated lymphocytes did not inhibit migration of macrophages from capillary tubes (Table 4). In contrast, supernatant fluids from a Candida skin test positive subject, prepared under identical conditions, reduced the area of migration by 48·5%. Media containing the Candida extract in a concentration equal to the culture fluids did not impair migration of the macrophages.

TABLE 4. Macrophage migration-inhibition factor activity*

| | Days following transfusion | | |
|---|---|---|---|
| | Prior to transfusion | 220 | 315 |
| | % inhibition | % inhibition | % inhibition |
| Patient | | | |
| Supernatants from: | | | |
| unstimulated cultures | 0 | 0 | 0 |
| MEM plus Candida extract | 0 | 0·7 | −14·6 |
| Candida stimulated cultures | 3·0 | 20·7 | 9·3 |
| Skin-Test Positive Control | | | |
| Supernantants from: | | | |
| unstimulated cultures | 0 | 0 | 0 |
| MEM plus Candida extract | 0 | 0 | −4·6 |
| Candida stimulated cultures | 48·5 | 20·5 | 28·9 |

* Each determination represents the mean of three chambers, each containing two capillary tubes.

An attempt to detect MIF activity in supernatants shortly after the leucocyte transfusion was a technical failure. When the study was repeated at 220 days, supernatants from Candida stimulated cells from the patient and control subject were equally effective in inhibiting migration from the capillary tubes. At 315 days, when the patient was in clinical relapse, and the delayed cutaneous reactions had reverted to negative, insignificant (9·3%) inhibition of migration was observed.

*Immunoglobulins and serological findings.* In contrast to abnormal cellular immunity, humoral responses of the patient were intact. The serum concentrations of the three major immunoglobulins were greater than normal (Table 5). Although IgE was not quantitatively

TABLE 5. Humoral immune responses and 'auto-antibodies' in the patient's serum

| Serum Immunoglobulins | Normal range |
|---|---|
| IgG–25·6 mg/ml | 5·0–12·0 γ/ml |
| IgA–7·2 mg/ml | 1·5– 4·0 γ/ml |
| IgM–3·6 mg/ml | 0·8– 3·0 γ/ml |
| IgE–present by skin test | |
| Candida Antibodies | |
| Serum agglutinins > 1:4096 | |
| Salivary agglutinins 1:16 | |
| Serum precipitins–positive | |
| Anti-A isoagglutinin 1:16 | |
| 'Auto-antibodies' | |
| anti-thyroglobulin 1:128 | |
| anti-thyroid C-F 1:4 | |
| anti-adrenal negative | |
| anti-gastric parietal cell negative | |
| rheumatoid factor (Latex) negative | |
| anti-nuclear factor negative | |
| cryoglobulin negative | |

assayed, the wheal and flare reactions to Candida and SK-SD indicated the presence and function of this substance. Serum and saliva contained high titres of agglutinating antibodies against *C. albicans* (Table 5), and the serum contained precipitins against both mannans and cytoplasmic components of the yeast. Antibody activities against thyroglobulin and thyroid cells were found in the serum, but no other serological abnormalities were present.

*Phagocytosis and inflammatory response.* There were no abnormalities in the ability of the patient's peripheral leucocytes to ingest and kill staphylococci or *C. albicans.* Furthermore, the metabolic responses following phagocytosis as measured by increased activity of the pentose pathway and oxidation of formate, were normal. The patient's serum was found to opsonize *C. albicans* normally and histochemical stain for leucocyte peroxidase was positive.

Evolution of the inflammatory response as measured by the skin window technique of Rebuck & Crowley (1955) was normal.

TABLE 6. Leucocyte typing

| | RBC Group | HL-A1 | -A2 | -A3 | -A9 | Lc17 | -A5 | -A7 | -A8 | HL-A12 | TE 60 (Fiske) | Lc20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | B | + | [+ 0] | 0 | 0 | [0 +] | 0 | + | 0 | 0 | [0 +] | + |
| Father | B | + | | 0 | 0 | | 0 | + | 0 | 0 | | + |
| Mother | A | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brother | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 |
| Sister | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | + | 0 |

* Mismatches involved in the leucocyte exchange are in square brackets.

*Leucocyte and erythrocyte typing.* The leucocyte antigens identified by the microdroplet cytotoxicity test are summarized on Table 6. None of the relatives were phenotypically identical with the patient. Furthermore, the mother and both siblings had different major erythrocyte antigens. The father, however, shared nineteen erythrocyte antigens with the patient, and possessed 's' which the patient lacked. Both leucocyte and erythrocyte cross-matches between the patient and his father were compatible.

Mixed leucocyte cultures also demonstrated the lack of HL-A identity in the family (Table 7). The magnitude of these responses was not altered when the cultures were incubated in autologous or homologous plasma. In the lower half of Table 7, the results of the reciprocal mixed leucocyte cultures are summarized and again brisk responses were found.

TABLE 7. Results of mixed leucocyte cultures

| Responding cells | Stimulating cells | | | |
|---|---|---|---|---|
| | Self | Father | Mother | Sister |
| Patient | 2170† | 16900 | 10300 | 31850 |
| Ratio* | 1 | 7·8 | 4·7 | 14·7 |

Reciprocal Stimulation

| Stimulating cells | Responding cells | | |
|---|---|---|---|
| | Father | Mother | Sister |
| Self | 1650 | 5180 | 2510 |
| Patient | 54950 | 83340 | 227650 |
| Ratio* | 33·3 | 16·1 | 90·7 |

* Mean dpm of response to allogenic cells divided by mean dpm of response to mitomycin treated autologous cells.

† Mean dpm from quadruplicate cultures.

*Clinical responses following leucocyte transfusions.* The evening following the third transfusion the patient became febrile and remained so for 48 hr. There were no other adverse effects.

During the ensuing months striking improvement was noted in the cutaneous lesions. This was especially apparent in areas that were not severely involved such as the scalp, neck (Fig. 1b), arms (Fig. 2b) and legs (Fig. 3b). More extensively affected areas such as the dorsum of the hands (Fig. 4b) also improved but to a lesser degree. There were no changes in the nails.

In the spring of 1970, approximately 8 months after the leucocyte transfusions, he noted recurrence of lesions in healed areas and exacerbation of areas with persistent infection. When he was studied in June, 1970, 10 months following the transfusions, the extent of the cutaneous lesions was similar to that of the previous year.

## DISCUSSION

Despite its widespread natural occurrence and frequent presence in the human gastrointestinal tract (Cohen *et al.*, 1970), severe infections due to *C. albicans* are uncommon. When systemic infections occur they are often in patients whose immune responses are impaired by malignancies (Hutter & Collins, 1962), immunosuppressive drugs (Rifkind *et al.*, 1967), or congenital defects of the thymus-dependent immune system such as the Di George syndrome, Nezeloff syndrome or lymphopenic agammaglobulinemia (Hermans *et al.*, 1969).

The mucocutaneous form of chronic moniliasis is unique in that it is limited to the mucous membranes, skin and nails, and essentially never involves parenchymal tissues. Reports containing several cases of this disorder illustrate the marked heterogeneity of the patient population in terms of age of onset, associated disorders such as polyendocrinopathy, steatorrhea, and dental dysplasia, and immunological responses, especially delayed cutaneous hypersensitivity (Sjoberg, 1966; Chilgren *et al.*, 1967; Louria *et al.*, 1967; Blizzard & Cibbs, 1968; Hermans *et al.*, 1969; Kirkpatrick *et al.*, 1970). In view of the clinical variants of mucocutaneous moniliasis, it is not surprising that extensive studies have failed to define a defect in host-defence mechanisms that characterized all patients.

Abnormalities in phagocytic or metabolic properties of the patient's leucocytes similar to those reported by Lehrer & Cline (1969) were excluded by the normal ingestion and killing of *C. albicans*, and the normal increase in oxidation of glucose and formate following phagocytosis.

Attempts to alter the course of systemic Candida infections in animals with active or passive immunization have produced variable results (Dobias, 1964). Patients with mucocutaneous moniliasis usually have normal or elevated titres of Candida antibodies in the serum and parotid fluid (Louria *et al.*, 1967; Kirkpatrick *et al.*, 1970) suggesting that humoral immune mechanisms are ineffective in resistance to this infection. A possible exception was described by Chilgren, and co-workers (1967), who reported two patients with mucocutaneous moniliasis and deficient Candida agglutinating antibodies in their parotid fluid IgA. These subjects also had negative cutaneous tests to Candida extracts, therefore their immunologic abnormality may involve the cellular immune system as well.

It is well known that addition of antigens to suspension cultures of sensitized peripheral blood lymphocytes induces biochemical and morphologic changes in a minor population of

the cells, and these *in vitro* responses correlate closely with delayed cutaneous hypersensitivity (Mills, 1966). When non-specific mitogens such as phytohemagglutinin or pokeweed mitogen are employed, the majority of the cells transform to 'blast-like' cells and undergo increased synthesis of DNA, RNA and protein (Ling, 1968). The cells that participate in these responses appear to be the 'thymus-dependent' small lymphocytes. Mitogen and antigen-induced lymphocyte transformation do not occur in cells from chicks that were thymectomized shortly after hatching (Greaves, Roitt & Rose, 1968), or with cells from infants born with a dysplastic or absent thymus (Lischner, Punnett & Di George, 1967).

Recently it has been shown that sensitized lymphocytes also respond to antigenic stimulation by releasing substances which inhibit migration of macrophages from capillary tubes (MIF) (David, 1968), are chemotactic for monocytes (Ward *et al.*, 1969), are cytotoxic (Granger & Williams, 1968) and recruit non-sensitized lymphocytes into DNA synthesis (Maini *et al.*, 1969; Kirkpatrick *et al.*, 1970). Injection of culture fluids containing MIF activity into the skin of normal animals produces histologic changes of delayed hypersensitivity (Bennett & Bloom, 1968) and it is generally believed that these 'mediators' are essential to the expression of cellular hypersensitivity.

Several reports of patients with chronic mucocutaneous moniliasis have noted negative delayed skin tests to *C. albicans* (Chilgren *et al.*, 1967; Louria *et al.*, 1967; Buckley *et al.*, 1968; Marmor & Barnett, 1968; Kirkpatrick *et al.*, 1970; Valdimarsson *et al.*, 1970). *In vitro* lymphocyte transformation studies have revealed that some patients with negative skin tests fail to respond to antigenic stimulation with an increase in thymidine incorporation (Chilgren *et al.*, 1967; Kirkpatrick *et al.*, 1970), while in others this response was normal (Chilgren *et al.*, 1967; Valdimarsson *et al.*, 1970). Chilgren *et al.* (1969) proposed that the latter group had a defect in production of mediators such as MIF. This hypothesis has been confirmed by Rocklin *et al.* (1970), Valdimarsson and co-workers (1970) and in the patient described in this report. Presumably these patients have antigen-sensitive peripheral blood lymphocytes that respond to *C. albicans* by replication, but are defective in the differentiative steps necessary for production or release of mediator substances.

The studies described here were directed toward defining the role of mediators in expression of delayed hypersensitivity, and evaluating the effect of an intact cellular immune system on the clinical course of chronic mucocutaneous moniliasis. It is clear that transfusions of large numbers of immunologically competent lymphoid cells were accompanied by conversion of the cutaneous response to *C. albicans* from negative to positive. During the months that the patient had reactive delayed cutaneous reactivity, supernatants from antigen-stimulated lymphocytes contained MIF activity. It is particularly significant that restoration of the immune response was accompaneid by clinical improvement. While the patient had normal delayed hypersensitivity and mediator production, there was marked clearing of the cutaneous lesions. Furthermore, when an exacerbation of moniliasis occurred eight months after the cell transfusions, the skin tests had reverted to negative and no MIF was found in supernatants.

Studies of antibody synthesis (Claman & Chaperon, 1969) and graft-versus-host disease (Cantor & Asofsky, 1970) in rodents have demonstrated the synergistic relationships of cells from two different sources. Although both cells are probably bone marrow-derived, one type receives a differentiative influence from the thymus and becomes an antigen-sensitive cell. In mice, the other bone marrow-derived cell is independent of the thymus and becomes the precursor of the antibody synthesizing cell. Little is known about the nature of the

interactions between these cells. Indeed, a mathematical analysis of the immune response to sheep erythrocytes in the mouse spleen has provided evidence for interactions involving three cell types (Mosier & Coppleson, 1968).

Studies of patients with chronic mucocutaneous moniliasis may provide evidence for multiple cell interactions in the expression of delayed hypersensitivity. For example, it is possible that a single antigen-sensitive peripheral lymphocyte responds to antigen by both mediator production and replication. Alternatively, the initial sensitizing experience may cause more primitive cells to differentiate into two lines, one that produces mediators in a manner analogous to antibody synthesis by plasma cells, and a second that responds by replication and serves to expand the pool of memory cells.

The findings in our patient could be explained by a defect in differentiation which did not affect cells with the capacity to replicate, but prohibited formation or function of the mediator producing cells. The transfusions of immuno-competent cells could have reconstituted this deficient or defective population and restored normal responsiveness. While it was not possible to document the duration of survival of the transfused allogenic cells, in man persistence of passively transferred delayed cutaneous hypersensitivity does not require the presence of viable donor cells. Lawrence (1969) has shown that 'transfer factor', released from sensitized lymphocytes by exposure to antigen or by lysis, is capable of transferring delayed cutaneous hypersensitivity specifically. Although not permanent, the transferred responses persist for months or years. The biological basis of its action is unknown. It is therefore possible that the cells transfused into our patient were rapidly destroyed and that the normal immune responses were mediated through transfer factor. Support for this possibility derives from the report of Rocklin *et al.* (1970), who found that MIF was produced by an anergic patient with moniliasis after treatment with transfer factor.

These studies support the earlier observations by Buckley *et al.* (1968) that reconstitution of the immune response may be useful in treating certain patients with chronic mucocutaneous moniliasis. It is important to recognize that this disorder may occur in several settings and not all cases have presently definable abnormalities of the immune system. Under ideal circumstances, correction of the defect in appropriate patients would utilize matched lymphocytes or bone marrow in order to minimize the possibility of graft-versus-host reactions, or rejection of the graft. Alternative modes of therapy such as transfer factor also deserve consideration because they may correct the immune defect without the risk of sensitizing the patient to histocompatibility antigens, or producing adverse immune responses.

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