

CHRONIC MUCOCUTANEOUS CANDIDIASIS, DERMATOPHYTOSIS AND DEFECTIVE CELLULAR IMMUNITY IN MONOZYGOTIC TWINS

H. Mobacken and L. Lindholm

*From the Department of Dermatology and the Institute of Medical Microbiology,
Department of General Bacteriology, University of Gothenburg, Gothenburg, Sweden*

Abstract. Two monozygotic 24-year-old brothers with chronic mucocutaneous candidiasis since the age of 8 have been studied. Skin infection due to *Epidermophyton floccosum* was also present in both, but otherwise they are not abnormally prone to infections. There is no endocrine dysfunction. Their cellular immunity is disturbed due to a qualitative lymphocyte defect, which manifests itself as an inability to be stimulated by antigens in vitro and possibly also as an inability to cause inhibition of leucocyte migration in vitro. It is suggested that the defect causing enhanced susceptibility to candida infection may also lead to a failure to resist dermatophytes.

Candida albicans is a common commensal in man. Acute infections are frequent and usually regress following treatment. Chronic candidiasis of the skin, nails and mucous membranes is rare, however; its resistance to therapy indicates a profound disturbance of the host-parasite relationship. Some such patients also present single or multiple abnormalities of the endocrine system, e.g. diabetes mellitus, hypoparathyroidism and adrenocortical insufficiency (16). This candida-endocrinopathy syndrome is inherited as an autosomal recessive trait (21). In other subjects, the disease is associated with profound and occasionally fatal immunologic deficiencies such as Di George's syndrome (7, 18, 20). There remains, however, a group of patients with chronic oral and cutaneous candidiasis without other clinical manifestations. The findings by Chilgren et al. (4) of defects in cellular immunity in that group of patients have subsequently been verified and extended (13, 21, 23). Predisposition to monilial infection may be genetically determined (23).

The discovery of the disease in a pair of identical twins is therefore interesting. We report a pair of identical twins who in addition to chronic mucocutaneous candidiasis (CMC) also suffer from wide-

spread dermatophytosis. Our findings in these patients suggest that immunity of the delayed hypersensitivity type may be of importance in man's resistance to dermatophytes.

CASE REPORTS

The twin brothers, B and K were born in 1949. The parents are unrelated, healthy and not aware of any skin diseases or abnormal tendency to infections in their relatives. As children, the boys were nearly identical in appearance and manners. In 1950 B had an appendectomy without complications. As children and at school they were BCG-vaccinated several times, but tuberculin (Mantoux) tests have always been negative. In 1957 they gradually developed soreness, erythema and white plaques in the oral cavity and the angles of the mouth. B also exhibited paronychia and nail deformities on 4 digits of the left hand. These symptoms have since persisted. Moniliasis was diagnosed, but local applications of gentian violet and nystatin were ineffective. In 1962, they were examined at the Department of Dermatology and the Children's Hospital, Gothenburg. *C. albicans* was again recovered from oral cultures and B's paronychia. In a biopsy specimen from the tongue, hyphae were seen penetrating the upper parts of the epithelium. Diabetes, hypothyroidism, hypoparathyroidism, adrenocortical insufficiency, vitamin and iron deficiencies were not found. Subsequently they have regularly been treated topically with gentian violet and nystatin in the mouth and on the paronychia as well as with several common topical antimycotics. Nothing seemed able to cure or even substantially alleviate. During the 1960s K had relapsing sinusitis, but B has had no apparent tendency to infections. In 1967, K had a severe local reaction and fever following smallpox primo-vaccination. During the past 2 years, they have been treated with amphotericin B lozenges (Fungilin®, Squibb) with fair results even if the monilial infection is not gone. Another antimycotic for topical use, Clotrimazol® (Bay b 5097, Bayer) was without effect.

In late puberty, they both violated the law and were found guilty of acts of violence and burglary, and were condemned to youth prison. Since then, each has lived on his own, but both have had difficulties in obeying the law and have been in prison again. They admit a high, regular ethanol con-

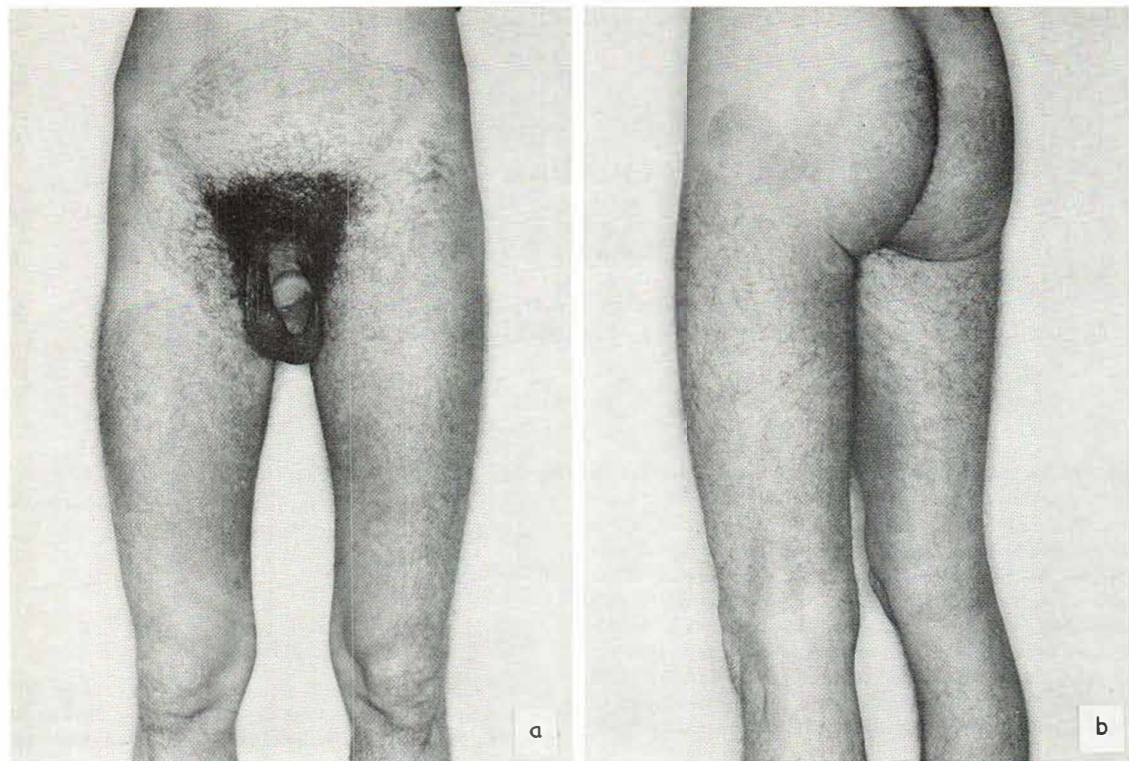


Fig. 1a, b. Widespread lesions from *Epidermophyton floccosum* (patient K).

sumption. Moreover, B has periodically taken narcotics, mostly by injection. He has also suffered 2 attacks of icteric hepatitis (1969 and 1971) which were attributed to drug abuse.

In 1968 B developed a red desquamating plaque on the inside of the right thigh and groin. Repeated cultures disclosed *Epidermophyton floccosum*. This has remained rather constant in spite of topical treatment with several antimycotics. In 1970 K acquired similar lesions that gradually spread from the groins to the abdomen, the lower part of the back, the thighs and lower legs (Fig. 1). Several fungal cultures produced *Epidermophyton floccosum*. Topical antimycotics did not influence the extensive fungous infection but systemic administration of griseofulvin in 1972 resulted in healing.

In 1970 and 1973 immunological function tests were performed. Physical examination on these occasions demonstrated that they both had white deposits, some of them easily detachable, on the buccal mucosa, palate, tongue and lips. Their tongues were enlarged and furrowed. Most of the teeth were severely carious. Bilaterally, cheilitis. B had redness and thickening of the nail wallon with onychodystrophy on four digits of the left hand. Their general clinical condition was satisfactory.

Laboratory findings

The results are valid for both the patients, unless otherwise stated. Complete blood cell count, calcium and phosphorus in the serum, serum electrolytes and serum creatinine: all

within normal limits. ESR 15 (K) and 6 (B) mm/hr. Blood group 0 Rh (+). Urine analysis, normal. X-rays of lungs, normal.

Circulating antibody production.

Antistreptolysin-0 and antistaphylolysin titres, normal. Wasserman, negative. Rubella antibody (haemagglutination-inhibition test): positive, titres 1:80 (B) and 1:60 (K). This was interpreted as probably persisting immunity after rubella infection. Serum complement-fixing *C. albicans* antibody, positive in titre 1:60. Immunofluorescent tests were negative for antibodies against thyroid, gastric parietal cells, cell nuclei, kidney and adrenals. Normal concentrations of immunoglobulins G, A, M and E in serum. Immunization against polio and typhoid-paratyphoid resulted in significantly raised antibody titres.

In vivo lymphocyte function tests

Intracutaneous tests read after 48 hours with 0.1 ml of PPD (2 TU/ml), Varidase® (streptokinase-streptodornase, Lederle, dil. 1:100), and a commercial preparation of *C. albicans* (0.5%, Bencard, Brentford, England), negative. Attempts to sensitize the brothers by topical exposure to 1-chloro-2,4-dinitrobenzene (DNCB) were made according to Aisenberg (1). They were challenged 3 weeks later with 0.1% DNCB in acetone. This technique enabled the sensitization of over 90% of healthy controls (14) but was unsuccessful in the brothers.

In vitro lymphocyte function tests

Lymphocyte stimulation tests were performed with the T-cell mitogens Phytohemagglutinin P (PHA) (Difco Laboratories, Detroit, Michigan) and Concanavalin A (Con A) (Miles-Yeda Ltd, Rehovoth, Israel), the B-cell mitogen Pokeweed Mitogen (PWM) (Grand Island Biological Company, Grand Island, New York) and the antigens PPD (Statens Seruminstitut, Copenhagen), *Monilia albicans* extract (Hollister-Stier Laboratories, Spokane, Wisconsin), Streptokinase-Streptodornase (Varidase®) (Lederle Laboratories, Wayne, New Jersey), Trichophyton extract (N.V. HAL Allergen Laboratory, Haarlem) and Mumps Skin Test Antigen (Lilly International Corporation, Indianapolis, Indiana).

For the stimulation tests, heparinized blood was diluted 8 times with Eagles medium containing 10% fetal calf serum, streptomycin 100 µg/ml and penicillin 100 IU/ml. 0.2 ml portions of diluted blood was cultured in the wells of Falcon Microtiter II® plates. Optimal amounts of mitogens or antigens were added in a volume of 10 µl. 2 µCi of ³H-thymidine (5 Ci/mmol, Radiochemical Centre, Amersham, Bucks) was added per well after 5 days of incubation at 37°. 18 hours after the addition of ³H-thymidine the cells were washed on Millipore filters. The filters were dried, dissolved in Soluene® (Packard Instrument Company, Downers Grove, Illinois) and counted in a Packard Series 3000 Liquid Scintillation Spectrophotometer using Permablend® (Packard Instrument Company) in toluene.

Quantitation of T and B lymphocytes in venous blood was performed according to the rosette formation tests described by Jondal et al. (12). Inhibition of leucocyte migration was performed using the polythene capillary tubing method of Hughes (11). The results of the tests are shown in Table I. It will be noted that the lymphocytes of both patients are, in the main, normally stimulated by all three mitogens, whereas stimulation with antigens is severely reduced or absent. The same result was obtained when cells were washed free from autologous serum prior to cultivation. The proportions of T and B lymphocytes are essentially normal in both patients. No inhibition of leucocyte migration in the presence of *Monilia albicans* extract was noted in patient B.

Iron studies

Neither of the brothers has had anemia. Following the report of Higgs & Wells (9) that many patients with CMC had depleted iron stores without frank anemia, one twin (K) was investigated for signs of latent iron deficiency. Serum iron concentration and iron-binding capacity, normal. Sternal marrow: normal sideroblasts count. The iron stores were somewhat reduced but within normal limits.

Chromosomal analysis

Chromosomal analysis made by the peripheral leucocyte culture technique, revealed male karyotypes. The chromosomes were normal in number and appearance.

Genetic studies

The twins have the same colour of hair and eyes and are of the same height. They were almost identical in appearance as children. Their parents are not related. Blood was examined for the blood group antigens AB0, Rh, Kell, Duffy, Lewis and MN and for the HL-A lymphocyte antigens. For

Table I. Results of lymphocyte function tests on patients B and K

Test	Patient B	Patient K
Lymphocyte stimulation with		
PHA	Normal	Normal
ConA	Normal	Normal
PWM	Normal	Slightly reduced
PPD	Absent	Weak
Candida	Absent	Weak
Trichophyton	Absent	Weak
Varidase	Absent	Absent
Mumps	Absent	Weak
% T lymphocytes	68	80
% B lymphocytes	25	21
Inhibition of leucocyte migration in the presence of <i>Monilia albicans</i> extract		
	No inhibition	Not done

all of these the twins were identical. This information reveals a $P < 0.05$ for dizygosity (3, 17).

DISCUSSION

Chronic mucocutaneous candidiasis is a rare disease. Wells et al. (23) only managed to collect 61 patients in England and Wales (population appr. 48 millions). Some of these patients exhibited the candida-endocrinopathy syndrome or belonged to a group with late-onset candidiasis. The remaining patients, who constituted a majority of the original group, could be separated into two clinicogenetic groups. Those in Wells' group I were not so severely affected. Affected sibs and consanguineous parents were common, thus indicating an autosomal recessive heredity. Our twins seem clinically to fit into this group, unless they develop endocrine disease. Onset of oral candidiasis may precede endocrinopathy by several years, but according to Lehner (16) endocrinopathy is seldom diagnosed after the age of 22 years. The occurrence of such a rare disease as CMC in more than one member of a family strengthens the possibility that a genetic factor is responsible for the lack of defence against *C. albicans* in these patients.

The nature of the defect which makes these patients unable to resist candida infections remains unclear. Studies of patients with immunodeficiency disease suggest that so-called cellular immune defence is essential for resistance to fungal disease (10). Defects in the cellular immune system have been described in CMC (4, 21). The lymphocyte dysfunction has not been uniform in these patients. The most common

immunological abnormality seems to be absence of cutaneous delayed reactivity to monilial antigen and a specific inability to produce Macrophage inhibitory factor (MIF) (5, 9, 21).

The studies performed on the two patients presented here support the existence of lymphocyte dysfunction in CMC. Both patients had normal amounts of T and B lymphocytes in the peripheral blood and their circulating lymphocytes showed normal responses to the three mitogens employed. There was, however, complete skin anergy in both patients to the antigens tested and in vitro lymphocyte response to antigens was severely reduced or absent. Furthermore, inhibition of leucocyte migration in the presence of candida antigen was not observed in the one patient tested. Both patients thus seem to possess a qualitative lymphocyte defect which manifests itself as an inability to be stimulated by antigens in vitro and possibly also as an inability to effectuate inhibition of leucocyte migration in vitro.

The skin anergy and depressed lymphocyte response in the twins were seen also with antigens other than candida antigen. It is therefore of interest that both patients also suffer from extensive dermatophyte infection. In the literature on CMC one finds several case reports of concurrent dermatophyte infections. These patients have some data in common: they have severe candida infection often with the formation of skin granulomas (group 2 according to Wells et al. (23), and the dermatophytosis is extensive and difficult to eradicate permanently. The dermatophytes isolated have been *Trichophyton tonsurans* (13), *Trichophyton purpureum* (2), *Microsporum canis* (22), *Microsporum audouini* (6) and *Epidermophyton floccosum* (15, 19). Two additional patients had an associated endocrine disease (Wells' group 3), hypothyreosis, and cultures from both showed the presence of *Epidermophyton floccosum* (13, 23). Reports of dermatophyte infections in patients who, like our twins, belong to group 1 of Wells' scheme are scarce and we have only managed to find one case (*Trichophyton rubrum*) (8). These findings suggest that the abnormality causing enhanced susceptibility to candida infection may also lead to a failure to resist dermatophytes.

Apart from their fungal infections, the patients described here have not been abnormally prone to infections. Judging from clinical data, our patients thus seem to have a defect in their infection immunity which is confined to infection with certain fungi. If

one assumes a correlation between depressed cellular immunity and infections, we feel that there is a discrepancy between the data on skin anergy and depressed lymphocyte response, and the lack of infections with other microorganisms than the two species of fungi. There is no general agreement, however, as to the causative correlation between lymphocyte abnormality and candida infections. Higgs & Wells (9) have recently suggested that it is secondary to the infection and that an abnormality in iron metabolism precedes the onset of CMC. A majority of their patients showed evidence of iron deficiency, and iron therapy produced clinical improvement.

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H. Mobacken, M.D.
 Department of Dermatology
 Sahlgrenska sjukhuset
 S-413 45 Gothenburg
 Sweden