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BRIEF COMMUNICATION

Chronic mucocutaneous candidiasis accompanied by enhanced antibody production

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SUMMARY

In four cases of chronic mucocutaneous candidiasis (CMCC) enhanced antibody production against candida antigen was detected in association with depressed cellular immunity. These patients showed extremely high agglutinin titre to candida antigen, while they were unable to mount delayed hypersensitivity reactions to candida antigen, tuberculin-purified protein derivative (PPD) or to dinitrochlorobenzene (DNCB). They also showed prolonged survival of skin homografts. There was no correlation between migration inhibitory factor (MIF) production and blast transformation in response to candida antigen.

INTRODUCTION

Although the opportunistic pathogen *Candida albicans* is commonly present in man, it rarely causes severe infection in otherwise healthy subjects. In many cases of candida infection, the disease is localized in the nails or skin. In a few cases, the disease becomes wide-spread on the mucosal membrane and on the skin, and even systemic throughout the visceral organs. When this occurs, the condition is called 'chronic mucocutaneous candidiasis' (CMCC). It most commonly occurs early in life and persists for many years. Recently, various immunological abnormalities have been demonstrated in patients with CMCC (Kirkpatrick, Rich & Bennet, 1971; Lehner, Wilton & Ivanyi, 1972; Valdimarsson *et al.*, 1973; Cahill, Ainbender & Glade, 1974). In many cases, cellular immunity is depressed not only against candida antigen, but also against other antigens. We have examined humoral and cellular immunity in five cases of CMCC. In four of them, enhanced antibody production against *C. albicans* was detected in association with depressed cellular immunity. The principal findings in these four cases are reported in this communication.

MATERIALS AND METHODS

Subjects. Five healthy control subjects, six patients with localized superficial candidiasis and four patients with CMCC were examined.

Assay of antibody against C. albicans. Titration of antibody against *C. albicans* was carried out by an agglutination method (Winner, 1955). Two-fold dilutions were made with 0·2 ml of phosphate-buffered saline (PBS) in Kahn's tubes. 0·2 ml of the suspension containing 3×10^7 formalin-fixed *C. albicans* per millilitre was added to each tube. The mixtures were kept at 4°C overnight. Agglutination was read macroscopically and confirmed microscopically.

Assay of skin reaction. Candida antigen for skin testing was prepared from *C. albicans* no. 24 kindly supplied by Dr Iwata, Tokyo University. The antigen preparation contained protein (50 μg/ml), RNA (17 μg/ml) and polysaccharide (5 μg/ml). Immediate and delayed skin reactions were read at 15 min and 24 hr respectively after the intradermal injection of 0·1 ml of candida antigen.

Delayed skin reactions were also examined 48 hr after the intradermal injection of PPD, a purified protein derivative obtained from the culture filtrate of tubercle bacilli.

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Assay of blast transformation. Examination of lymphocyte-blast transformation in the presence of candida antigen (5 μg protein/ml) or a lectin PHA-P (1 μg/ml, Wellcome) was carried out by the method of Valdimarsson *et al.* (1973) after minor modifications. Blast transformation was measured by increased uptake of tritiated thymidine by peripheral blood lymphocytes. The response to candida antigen was expressed by a stimulation index and the response to PHA in the patients with CMCC was compared with the average value in healthy subjects.

Assay of migration inhibitory factor. 2×10^6 Peripheral blood lymphocytes per millilitre were incubated for 24 hr in the presence of candida antigen (5 μg protein/ml) in order to obtain the supernatant containing migration inhibitory factor (MIF). The supernatant used as the culture medium in which the migration of peritoneal macrophages from normal guinea-pigs was examined.

Assay of DNCB sensitization. In three cases of CMCC sensitization by dinitrochlorobenzene (DNCB) was performed. For sensitization, 0·025 ml of 2% solution of DNCB in acetone was applied with an adhesive plaster to the skin of one forearm for 48 hr. Two weeks later, 0·025 ml of 0·1% solution of DNCB in acetone was applied with an adhesive plaster to the skin of the opposite forearm. A positive response was recorded if erythema with induration was detected 48 hr later.

Assay of homograft immunity. In two patients, homotransplantation of skin was made and the survival time of the graft was recorded.

Assay of virulence of C. albicans isolated from patients. Nine strains of *C. albicans* isolated from five localized candidiasis cases and four CMCC cases were tested for their virulence to mice. 5×10^6 Cells of each strain in 0·5 ml were inoculated intravenously into seven mice of SL strain and the survival time of the mice was recorded.

RESULTS

Agglutinin titres to *C. albicans*, immediate and delayed skin reactions to candida antigen and delayed skin reaction to PPD were examined in healthy subjects, in the patients with localized superficial candidiasis and in the patients with CMCC. Agglutinating antibody was not detectable in the healthy subjects and negative or only low titres of antibody were found in the cases of localized candidiasis. On the contrary, agglutinating antibody of high titre was detected in all the cases of CMCC. Three of them showed extremely high titres.

TABLE 1. Immune responses in healthy subjects, the patients with localized superficial candidiasis and the patients with CMCC

Groups*	Agglutinin titres to *C. albicans*	Skin reaction to candida antigen†		Delayed skin reaction to PPD (48 hr)
		Immediate (15 min)	Delayed (24 hr)	
Healthy subjects	< 12·5	5×5	0×0	11×15
	< 12·5	5×5	0×0	5×5
	< 12·5	15×15	4×4	12×11
	< 12·5	30×30	0×0	11×10
	< 12·5	5×5	0×0	13×13
Localized superficial candidiasis	< 12·5	20×25	11×11	0×0
	< 12·5	10×10	7×7	2×3
	< 12·5	22×18	12×10	12×10
	25	27×22	6×6	0×0
	12·5	5×5	8×8	16×16
	< 12·5	15×15	12×15	10×10
CMCC‡	200	22×18	3×3	0×0
	200	25×25	0×0	0×0
	200	31×31	6×7	10×8
	50	25×25	3×3	0×0

* Groups of healthy subjects and localized candidiasis included the persons from 15 to 37 years of age.

† The longest multiplied by the shortest diameter of erythema (mm).

‡ Chronic mucocutaneous candidiasis.

TABLE 2. Detailed findings of the cases of choronic mucocutaneous candidiasis (CMCC)

Age	Sex	Age at onset	Infected sites	Associated abnormalities	Immunoglobulin (mg/dl)		Blast transformation		MIF production to candida	DNCB sensitization	Survival days of skin homografts
							Candida*	PHA†			
18	M	5	Buccal tongue nails skin	Tinea verrucosa Cryptococcal meningitis	IgG IgM IgA	2320 128 360	n.d.	n.d.	n.d.	Negative	24
27	M	25	Buccal tongue pharynx larynx nails skin	Liver cirrhosis	IgG IgM IgA	2600 90 700	0·8	Normal	Slightly positive	Negative	n.d.
14	M	3	Buccal nails skin	Idiopathic hypoparathyroidism	IgG IgM IgA	920 120 80	2·8	Depressed	Slightly positive	Negative	28
4	F	1	Buccal tongue nails skin	Not detectable	IgG IgM IgA	2340 74 190	12·4	Normal	Negative	n.d.	n.d.

n.d. = Not determined.

* Stimulation index was calculated by the formula: $\frac{[^3H]\text{thymidine uptake in the presence of antigen}}{[^3H]\text{thymidine uptake in the absence of antigen}}$.

† Compared with average value in healthy subjects.

An immediate skin reaction to candida antigen was detectable in most of the subjects of the three groups. Although all the cases of CMCC with high antibody titre exhibited strong immediate reactions, the degree of skin reaction did not correlate with the titre of agglutinating antibody in the other two groups. Delayed skin tests to candida antigen were positive in all the patients with localized candidiasis, while negative or weak reactions were found in the healthy subjects and in the patients with CMCC. Delayed skin tests to PPD were positive in many of the healthy subjects and in the patients with localized candidiasis, while only one of four patients with CMCC exhibited a slightly positive reaction.

Table 2 shows detailed findings in addition to those presented in Table 1 in the patients with CMCC. Onset of the infection was early in life in three of them. In all cases the infection had spread on to the buccal mucosa, tongue, nails and skin. The serum level of IgG was increased in three of them. Blast transformation and MIF production in response to candida antigen were dissociated in all cases so far examined. Sensitization by DNCB failed in all CMCC cases examined (cases 1, 2 and 3). Both cases of skin homotransplantation (case 1 and 3) showed prolonged graft survival.

C. albicans isolated from the patients with CMCC showed the same degree of virulence in mice as did those isolated from the patients with localized candidiasis.

DISCUSSION

Uptake and digestion by non-immune phagocytes, thymus-derived (T) cell-mediated cellular immunity in which activated macrophages work as direct effectors, and antibody-mediated mechanisms have been implicated in the body's defence against microorganisms. The relative importance of these mechanisms to protection against *C. albicans* have not yet been determined.

From the present findings, however, antibody of high titre appears to be incapable of eliminating *C.*

albicans in the patients with CMCC. Antibody may be ruled out at least as the main effector in the elimination of *C. albicans*, although the exact role of antibody remains uncertain as indicated by Axelsen, Kirkpatrick & Buckley (1974).

The patterns of depression of cellular responses to candida antigen, PPD and PHA were diverse among the cases of CMCC as shown by Valdimarsson *et al.* (1973).

We should like to propose that the subjects prone to CMCC may all have some degree of deficiency in T-cell function. Their T cells may work well enough as helper cells in antibody production, but not well enough to develop fully cellular immunity. The persistent existence of *C. albicans* facilitates antibody production in the presence of partially depressed T cell functions. Depression of cellular protective immunity may render the hosts incapable of eliminating *C. albicans* completely. On the other hand, presumed normal function of phagocytes may prevent the spread of infection in the patients with CMCC.

Enhanced antibody production associated with depressed cellular immunity has also been reported in lepromatous leprosy by Turk & Bryceson (1971). In the lepromatous leprosy and CMCC, the immune response appears to be deficient in the development of cellular immunity compared to antibody production. The importance of this to the establishment of long-standing infections may not be fully understood until detailed studies on the contribution of phagocytes in relation to T cell-mediated cellular immunity in the defence against such micro-organisms has been achieved.

REFERENCES

Axelsen, H.H., Kirkpatrick, C.H. & Buckley, R.H. (1974) Precipitins to *Candida albicans* in chronic mucocutaneous candidiasis studied by crossed immunoelectrophoresis with intermediate gel. Correlation with clinical and immunological findings. *Clin. exp. Immunol.* **17**, 385.

Cahill, L.T., Ainbender, E. & Glade, P.R. (1974) Chronic mucocutaneous candidiasis: T cell deficiency associated with B cell dysfunction in man. *Cell. Immunol.* **14**, 215.

Kirkpatrick, C.H., Rich, R.R. & Bennet, J.E. (1971) Chronic mucocutaneous candidiasis: model-building in cellular immunity. *Ann. intern. Med.* **74**, 955.

Lehner, T., Wilton, J.M.A. & Ivanyi, L. (1972) Immunodeficiencies in chronic muco-cutaneous candidiasis. *Immunology*, **22**, 775.

Turk, J.L. & Bryceson, A.D.M. (1971) Immunological phenomena in leprosy and related diseases. *Advanc. Immunol.* **13**, 209.

Valdimarsson, H., Higgs, J.M., Wells, R.S., Yamamura, M., Hobbs, J.R. & Holt, P.J.L. (1973) Immune abnormalities associated with chronic mucocutaneous candidiasis. *Cell. Immunol.* **6**, 348.

Winner, H.I. (1955) A study of *Candida albicans* agglutinins in human sera. *J. Hyg. (Camb.)*, 509.