Clin. exp. Immunol. (1978) **34**, 311–317.

Plasma thymic hormone activity in patients with chronic mucocutaneous candidiasis

C. H. KIRKPATRICK, LYNN E. GREENBERG, S. W. CHAPMAN, G. GOLDSTEIN, VERNA M. LEWIS & J. J. TWOMEY, *Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, USA, Ortho Pharmaceutical Corporation, Raritan, New Jersey, USA, and Immunohematology Research Laboratory, Veterans Administration Hospital, Houston, Texas, USA*

(*Received* 24 *May* 1978)

SUMMARY

To further characterize the immunological abnormalities in patients with chronic mucocutaneous candidiasis, the thymic hormone activity in their plasma was measured. Of the sixteen patients in the study, seven had chronic diffuse candidiasis, five had candidiasis with endocrinopathies and four had candidiasis with thymoma. Only one patient, an anergic child with chronic diffuse candidiasis had severe deficiency of plasma thymic hormone activity. Two patients, a woman with candidiasis and multiple endocrinopathies and an elderly man with metastatic epithelial thymoma had supranormal values.

These studies indicate that the immunological deficit in most patients with these forms of chronic mucocutaneous candidiasis is not due to deficiency of a thymic inductive activity and suggest that an intrinsic defect exists in the maturation of antigen-responsive lymphoid cells.

INTRODUCTION

Chronic mucocutaneous candidiasis is a persistent infection of the skin, nails and mucous membranes with candida species, usually *Candida albicans*. The disorder may occur in a variety of clinical settings including defects in the maturation of cell-mediated immune responses (Hermans, Ulrich & Markowitz, 1969; Kirkpatrick, Rich & Bennett, 1971; Lehner, Wilton & Ivanyi, 1972; Valdimarsson *et al.*, 1973), patients with 'autoimmune' endocrinopathies such as hypoparathyroidism, hypoadrenalism and hypothyroidism (Louria *et al.*, 1967; Blizzard & Gibbs, 1968) and in adults with thymomas (Montes *et al.*, 1972). Abnormalities of the thymus-dependent immune system are found in the majority of patients with chronic mucocutaneous candidiasis, although the manifestations and the severity of the defects are not the same in all patients.

In an attempt to define more completely the immunological functions in patients with this disorder and to identify patients who might be candidates for therapy with thymic hormones, we have measured the activity of this substance in the plasmas of sixteen patients with chronic diffuse candidiasis (Lehner, 1966), the candidiasis–endocrinopathy syndrome or the candidasis–thymoma syndrome. Severely deficient activity was found in only one patient, even though the majority of patients were known to have defects in expression of cell-mediated immunity to candida.

MATERIALS AND METHODS

Clinical material. Serum or plasma from sixteen candidiasis patients was studied. Cases 1–7 had chronic diffuse mucocutaneous candidiasis, cases 8–12 had candidiasis and deficient function of one or more endocrine organs (Table 1). Cases 13–16 had candidiasis with thymoma (Table 2). With the exception of case 14, all patients were evaluated at the NIH

Correspondence: Dr C. H. Kirkpatrick, Building 10, Room 11B-13, National Institutes of Health, Bethesda, Maryland 20014, USA.

0099-9104/78/0120-0311$02.00

TABLE 1. Patients with chronic diffuse candidiasis or candidiasis with endocrinopathy

Case no.	Age/Sex	Age onset candidiasis (yrs)	Candida skin test (cm indur)	T cells percentage (± s.e.)	Lymphocyte responses to candida: Lymphocyte transformation (Control DPM)	(s.r.)*	Lymphokine production (percentage inhibition)	Plasma thymopoietin equivalent (ng/ml)
A. Chronic diffuse candidiasis								
1	5 WF	4/12	0†	46·0 ± 8·0	750	0·9	n.t.	0, 1·4
2	16 WM	8/12	0	n.t.*	2180	1·9	n.t.	16·0, 15·2
3	14 WM	3	0	48·0 ± 6·8	625	0·4	0	10·2
4	10 WM	3	0	41·5 ± 3·6	1100	11·0	2·0	10·6
5	18 WF	6	0	51·5 ± 4·6	4590	1·8	2·0	12·6
6	28 WF	6	0·5§	61·6 ± 5·8	5325	0·9	42·6	10·8
7	23 WF	10	1·2	64·0 ± 2·6	647	6·1	3·9	12·6
B. Chronic candidiasis with endocrinopathy								
8	26 WM	9/12	1·2§	67·5 ± 1·5	3435	5·6	16·8	13·2
9	37 WF	2	0·7§	64·0 ± 1·2	5500	5·2	19·7	19·4
10	12 WF	3	0†	65·5 ± 8·6	982	5·9	n.t.	10·6, 10·2
11	11 WM	3	0·7	58·0 ± 6·2	6000	8·8	n.t.	10·0, 10·8
12	12 WM	4	1·2	64·6 ± 5·0	2650	7·9	n.t.	10·8
Normal values				40–75		4·5–25·0	⩾15	

* s.r. = Stimulation ratio; n.t. = not tested

† Anergic patients

§ Skin reactivities after transfer factor therapy

TABLE 2. Patients with chronic mucocutaneous candidiasis and thymoma

Case no.	Age/Sex	Age onset: Candidiasis (yrs)	Age onset: Thymoma (yrs)	Cell type	Candida skin test (cm indur)	T cells (percentage ± s.e.)	Lymphocyte responses to candida: Lymphocyte transformation (Control DPM)	(s.r.)	Lymphokine production (percentage inhibition)	Plasma thymopoietin equivalent (ng/ml)
13	40 WF	36	36	Mixed	0	70·0 ± 7·2	2775	1·3	n.t.	12·6
14	40 WF	38	40	Mixed	positive	n.t.	normal		n.t.	5·2
15	56 WF*	50	38	Benign Lymphoid	0	74·00 ± 2·3	1332	1·7	n.t.	7·4
16	66 WM*	66	53	Malignant Epithelial	0·9	58·2 ± 4·0	3060	11·2	16·0	16·0, 12·6, 9·0

* Patients with myasthenia gravis

Clinical Center where studies of immunological functions were performed. The serum and a resumé of the immunological findings on case 14 were provided by Dr Dorothy Windhorst. The patients with either chronic diffuse candidiasis or the candidiasis–endocrinopathy syndrome developed the infection during infancy or early childhood. In contrast, the thymoma patients were not susceptible to infections with candida or other organisms until adulthood.

Immunological studies. All *in vitro* studies of lymphocyte function were performed on Hypaque–Ficoll purified, thrice washed peripheral blood mononuclear cells (Böyum, 1968).

The percentage of thymus-derived T lymphocytes was determined by the sheep erythrocyte rosette method of Jondal, Holm & Wigzell (1968). Immunoglobulin-bearing B lymphocytes were detected with a fluorescein-labelled goat anti-human immunoglobulin antiserum (Meloy Laboratories, Springfield, Virginia) and a Zeiss fluorescence microscope that was equipped for epi-illumination to facilitate identification of monocytes. Normal values were obtained from normal subjects studied concurrently.

Lymphocyte transformation responses to phytohaemagglutinin (Burroughs Wellcome, Research Triangle Park, North Carolina), concanavalin A (Miles Laboratories, Elkhart, Indiana), pokeweed mitogen (GIBCO, Grand Island, New York), streptokinase–streptodornase (Lederle Laboratories, Pearl River, New York) and *Candida albicans* (Hollister-Stier Laboratories, Spokane, Washington) were performed as described previously (Kirkpatrick *et al.*, 1976). Briefly, peripheral blood lymphocytes were suspended in RPMI 1640 with 10% plasma and incubated with the appropriate concentrations of antigens or mitogens at 37°C in a water-saturated atmosphere of 5% carbon dioxide in air. Mitogen-stimulated cultures were labelled with tritiated thymidine and harvested on the third day; antigen-stimulated cultures were labelled and harvested on day 5. In most instances, the responses were studied in the presence of autologous and group AB homologous plasma.

Production of macrophage migration inhibition factor (MIF) by antigen-stimulated lymphocytes was assessed according to the indirect method of Rocklin, Meyers & David (1970). Production of leucocyte migration inhibition factor (LMIF) by antigen or mitogen-stimulated lymphocytes was measured by our two-step method (Chapman & Kirkpatrick, 1978) that was derived from the method of Clausen (1973).

Normal subjects with positive or negative delayed cutaneous reactions to candida provided the normal values for the *in vitro* responses by lymphocytes. Delayed skin responses to *C. albicans*, streptokinase–streptodornase, PPD, trichophytin and tetanus toxoid were assessed as described previously (Kirkpatrick *et al.*, 1976). Positive responses produced indurations of 0·5 cm or greater at 24–48 hr.

Assay of thymic hormone activity. The bioassay for thymic hormone activity measures Thy 1·2 antigen induction on null mouse lymphocytes and has been described in detail elsewhere (Twomey *et al.*, 1977). Briefly, heparinized plasma samples, collected in close proximity to the lymphocyte function studies and stored at −70°C, were first filtered through Diaflo PM 30 membranes (Amicon Corporation, Lexington, Massachusetts) to remove non-specific toxic substances. Indicator cells were obtained from the spleens of germ-free nude mice outbred on a C3H/He background. Macrophages and B lymphocytes were depleted by incubation of the cells on a nylon-wool column. Induction incubations were in 0·2 ml and contained $0{\cdot}5 \times 10^6$ indicator cells, 125 μg per ml of ubiquitin which inhibits non-specific T-cell induction, and 0·05 ml test plasma filtrate. Incubations were for 18 hr at 37°C. Thy 1·2 antigen induction was measured using a specific antiserum (Reif & Allen, 1964), guinea-pig complement and a highly sensitive enzymatic cytotoxicity test. Optimal induction for individual indicator cell preparations was determined from incubations that included 1 μg thymopoietin. The relationship between the percentage of cells induced to express Thy 1·2 and concentration of the thymopoietin standard is linear. This permits expression of results as being equivalent in activity to known amounts of thymopoietin.

RESULTS

Immunological responses

None of the sixteen subjects of this study was lymphopenic and all persons had normal percentages of circulating immunoglobulin-bearing B lymphocytes.

In our laboratory, 40–75% of peripheral blood lymphocytes from normal subjects form spontaneous rosettes with sheep erythrocytes (T lymphocytes). As shown in Tables 1 and 2, none of the patients was deficient in T cells, although case 4 was at the lower edge of our normal range.

Delayed cutaneous hypersensitivity responses to candida were absent in five of the seven patients with chronic diffuse candidiasis, one of the five patients with the candidiasis–endocrinopathy syndrome and two of the four candidiasis–thymoma patients. Two patients (cases 1 and 10) were unresponsive to all of the test antigens. Three additional patients (cases 6, 8 and 9) had negative candida skin tests when they were first evaluated in our laboratory, but at the time the plasma samples were obtained they were receiving periodic injections of transfer factor and had acquired the ability to express delayed hypersensitivity to candida.

Every subject of this study had normal lymphocyte transformation responses to phytohaemagglutinin, concanavalin A and pokeweed mitogen.

In our laboratory, stimulation of peripheral blood lymphocytes from candida-sensitive normal subjects with candida antigen produces 4·5–25-fold increases in thymidine incorporation. Skin-test-negative normal subjects have either no responses or small (three-fold) increases. Seven of the sixteen patients with chronic mucocutaneous candidiasis had subnormal lymphoproliferative responses to candida antigen. It is noteworthy that in these patients an exact correlation between delayed cutaneous reactions and *in vitro* lymphocyte responses to antigens (especially candida) was not always found. This is illustrated by the data in Table 1 in which cases 4 and 10 had negative candida skin tests, but normal lymphocyte transformation responses, and case 6 had a positive candida skin test (after receiving transfer factor), but failed to develop proliferative responses to candida. These results could not be changed by culturing the patients' cells in normal plasma. With the thymoma patients, there was concordance between the delayed cutaneous and lymphocyte transformation responses to candida.

Lymphokine production in response to concanavalin A and candida was studied in eight patients. In each case, concanavalin A-stimulated cells produced supernatant fluids that inhibited migration of the indicator cells by at least 15%. These results are comparable to those of normal lymphocytes (Chapman & Kirkpatrick, 1978) and demonstrated that the patients had cells that were potentially able to produce LMIF or MIF. However, four patients with chronic diffuse candidiasis produced subnormal amounts of MIF in response to candida. Three of these patients also had negative skin tests and two had subnormal lymphoproliferative responses to candida. Four other patients manifested both delayed hypersensitivity and MIF responses. Thus, delayed hypersensitivity showed greater concordance with lymphokine production than with lymphoproliferative responses to this antigen.

Thymopoietin-like activity

The 95% confidence limits of the normal range for plasma thymic hormone activity for healthy subjects of various ages are shown in Fig. 1. The highest concentrations are found during the second and third age decades. After the fifth decade, there is a rapid fall in plasma activity and by the seventh decade virtually no activity is detectable.

Only one of the candidiasis patients (case 1) was severely deficient in circulating thymopoietin-like activity. This child was anergic to all five antigens and her lymphocytes failed to proliferate when cultured with any antigens. Seven other patients had values close to the lower limit of the normal range. Case 16, a 68-year-old male with a progressive malignant epithelial thymoma, had elevated plasma thymic

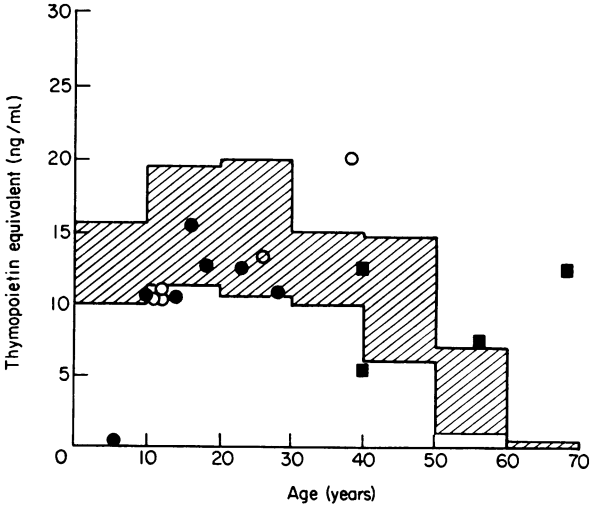

FIG. 1. Plasma thymic hormone activity in patients with mucocutaneous candidiasis. Note that only one patient had severe deficiency and several additional patients were at the lower limits of normal. Two patients had elevated values. The hatched zone defines the 95% confidence limits for each decade. (●) Chronic diffuse candidiasis; (○) candidiasis with endocrinopathy and (■) candidiasis with thymoma.

hormone activity on three separate determinations. However, three other patients with thymomas did not have elevated levels. Case 9, a 37-year-old female who developed candidiasis in childhood and had multiple endocrinopathies had abnormally high plasma thymic hormone activity in the absence of a detectable thymoma.

DISCUSSION

The syndrome of chronic mucocutaneous candidiasis may occur in a variety of clinical settings. Several reports have shown that the majority of patients with the disorder have defects in cell-mediated immunity that may be either limited to the antigens of candida or involve a broad spectrum of antigens (Kirkpatrick & Montes, 1974; Lehner, Wilton & Ivanyi, 1972; Valdimarsson *et al.*, 1973), and it is generally believed that the immunological defects predispose the patients to infection with this opportunistic organism. Two lines of evidence provide support for this model. Firstly, clinically similar disorders occur in patients with well-defined immunodeficiency syndromes such as severe combined immunodeficiency, the Di George syndrome or the thymic hypoplasias (Hermans *et al.*, 1969). Secondly, several candidiasis patients have received clinical benefits from reconstitutive procedures such as grafts of thymus tissue (Levy *et al.*, 1971), transfusions of leucocytes from candida-immune donors (Kirkpatrick *et al.*, 1971; Valdimarsson *et al.*, 1972), administration of transfer factor (Kirkpatrick & Smith, 1974; Spitler *et al.*, 1975) or thymus extracts (Wara & Ammann, manuscript to be published) or combined therapy (Kirkpatrick *et al.*, 1976; Ballow & Hyman, 1977).

The heterogeneity of chronic candidiasis is further illustrated by patients with candidiasis and thymoma about whom there is little immunological information, and the recently reported candidiasis patients who have abnormal monocyte functions (Snyderman *et al.*, 1973; Twomey *et al.*, 1975). Parenthetically, the recent report (Twomey, Lazar & Rocklin, 1977) that MIF production by normal lymphocytes does not require a contribution from monocytes should provide an additional means for identifying the fundamental cellular defects in candidiasis patients.

Deficits of T-cell function could result from inadequate inductive stimuli from the thymus with qualitative and/or quantitative deficiencies or from intrinsic differentiative defects in the T cells themselves. Optimal results from reconstitutive procedures should depend on the precise identification of the immunological defect in each patient. It was to this end that the present study was undertaken. A profound deficiency of thymic hormone activity was found in only one patient, although eleven of the sixteen patients were known to have defective cell-mediated immunity. Since this assay clearly detects subnormal levels of thymic hormone in classic immunodeficiency syndromes (Lewis *et al.*, 1977), the cellular immune abnormalities in the majority of candidiasis patients in the present study cannot be attributed to deficiencies of thymic hormone. Therapy with thymic hormone could be considered, but this would constitute an attempt at the modulation of the immune system rather than the replacement of a deficient induction factor.

Four of the sixteen patients had thymomas, but only the patient with an epithelial thymoma and myasthenia gravis had elevated plasma thymic hormone activity. This may be related to the fact that thymic hormone is synthesized in epithelial elements of the normal thymus (Goldstein, 1975a); or to the associated autoimmune thymitis and myasthenia gravis (Goldstein, 1975b). It also suggests that the candidiasis and immunodeficiency that is associated with thymoma is not due to deficient thymic hormone activity. Additional patients must be studied to determine if elevated plasma thymic hormone will identify residual or active tumours in patients with epithelial thymomas.

The authors are grateful to Mrs Marilyn Schoenfelder for preparation of the manuscript. This research was supported in part by USPHS grant CA 19538 and a grant from the Kelsey and Leary Foundation #970.

REFERENCES

Ballow, M. & Hyman, L.R. (1977) Combination immunotherapy in chronic mucocutaneous candidiasis. Synergism between transfer factor and fetal thymus tissue. *Clin. Immunol. Immunopath.* **8**, 504.

BLIZZARD, R.M. & GIBBS, J.H. (1968) Candidiasis: Studies pertaining to its association with endocrinopathies and pernicious anemia. *Pediatrics*, **42**, 231.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. clin. Lab. Invest.* **21**, supplement 97, 77.

CHAPMAN, S.W. & KIRKPATRICK, C.H. (1978) The two-step leukocyte migration inhibition factor (LIF) assay: Its use in elevation of cellular immune function in patients with immunodeficiency diseases. *Cell. Immunol.* **37**, 209.

CLAUSEN, J.E. (1973) Migration inhibitory effect of cell-free supernatants from tuberculin-stimulated cultures of human mononuclear leucocytes demonstrated by two-step MIF agarose assay. *J. Immunol.* **110**, 546.

GOLDSTEIN, G. (1975a) The isolation of thymopoietin (thymin). *Ann. N.Y. Acad. Sci.* **249**, 17.

GOLDSTEIN, G. (1975b) 'Myasthenia gravis'—or the autoimmunity, thymic disease, neuromuscular blockade poser. (Editorial.) *West. J. Med.* **123**, 138.

HERMANS, P.E., ULRICH, J.A. & MARKOWITZ, H. (1969) Chronic mucocutaneous candidiasis as a surface expression of deep-seated abnormalities. *Amer. J. Med.* **47**, 503.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. A large population of lymphocytes forming non-immune rosettes with sheep and blood cells. *J. exp. Med.* **136**, 207.

KIRKPATRICK, C.H. & MONTES, L.F. (1974) Chronic mucocutaneous candidiasis. *J. Cutan. Path.* **1**, 211.

KIRKPATRICK, C.H., OTTESEN, E.A., SMITH, T.K., WELLS, S.A. & BURDICK, J.F. (1976) Reconstitution of defective cellular immunity with foetal thymus and dialysable transfer factor. *Clin. exp. Immunol.* **23**, 414.

KIRKPATRICK, C.H., RICH, R.R. & BENNETT, J.E. (1971) Chronic mucocutaneous candidiasis: Model-building in cellular immunity. *Ann. Int. Med.* **74**, 955.

KIRKPATRICK, C.H., RICH, R.R., GRAW, R.G., SMITH, T.K., MICKENBERG, I.D. & ROGENTINE, G.N. (1971) Treatment of chronic mucocutaneous moniliasis by immunologic reconstitution. *Clin. exp. Immunol.* **9**, 733.

KIRKPATRICK, C.H. & SMITH, T.K. (1974) Chronic mucocutaneous candidiasis: Immunologic and antibiotic therapy. *Ann. Int. Med.* **80**, 310.

LEHNER, T. (1966) Classification and clinico-pathological features of candida infections in the mouth. *Candida Infections* (ed. by H. I. Winner and R. Hurley), p. 119. Williams & Wilkins, Co., Baltimore.

LEHNER, T., WILTON, J.M.A. & IVANYI, L. (1972) Immunodeficiencies in chronic mucocutaneous candidiasis. *Immunology*, **22**, 775.

LEVY, R.L., HUANG, S-W., BACH, M.L., BACH, F.H., HONG, R., AMMANN, A.J., BORTIN, M. & KAY, H.E.M. (1971) Thymic transplantation in a case of chronic mucocutaneous candidiasis. *Lancet*, ii, 898.

LEWIS, V., TWOMEY, J.J., GOLDSTEIN, G., O'REILLY, R., SMITHWICK, E., PAHWA, R., PAHWA, S., GOOD, R.A., SCHULTE-WISSERMAN, H., HOROWITZ, S., HONG, R., JONES, J., SIEBER, O., KIRKPATRICK, C., POLMAR, S. & BEALMEAR, P. (1977) Circulating thymic hormone activity in congenital immunodeficiency. *Lancet*, ii, 471.

LOURIA, D.B., SHANNON, D., JOHNSON, G., CAROLINE, L., OKAS, A. & TASCHDJIAN, C. (1967) The susceptibility to moniliasis on children with endocrine hypofunction. *Trans. Ass. Am. Physic.* **80**, 236.

MONTES, L.F., CEBALLOS, R., COOPER, M.D., BRADLEY, M.N. & BOCKMAN, D.E. (1972) Chronic mucocutaneous candidiasis, myositis and thymoma. *JAMA*, **222**, 1619.

REIF, A.E. & ALLEN, J.M.V. (1964) The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. exp. Med.* **120**, 413.

ROCKLIN, R.E., MEYERS, O.L. & DAVID, J.R. (1970) An *in vitro* assay for cellular hypersensitivity in man. *J. Immunol.* **104**, 95.

SNYDERMAN, R., ALTMAN, L.C., FRANKEL, A. & BLAESE, 1973) Defective mononuclear leukocyte chemotaxis: A previously unrecognized immune dysfunction. *Ann. Int. Med.* **78**, 509.

SPITLER, L.E., LEVIN, A.S. & FUDENBERG, H.H. (1975) Transfer factor II. Results of therapy. *Birth Defects Orig. Art. Ser.* **11**, 449.

TWOMEY, J.J., WADDELL, C.C., KRANTZ, S., O'REILLY, R., L'ESPERANCE, P. & GOOD, R.A. (1975) Chronic mucocutaneous candidiasis with macrophage dysfunction, a plasma inhibitor and co-existent aplastic anemia. *J. Lab. clin. Med.* **85**, 968.

TWOMEY, J.J., GOLDSTEIN, G., LEWIS, V.M., BEALMEAR, P.M. & GOOD, R.A. (1977) Bioassay determinations of thymopoietin and thymic hormone levels in human plasma. *Proc. Nat. Acad. Sci. (Wash.)*, **74**, 2541.

TWOMEY, J.J., LAZAR, S. & ROCKLIN, R.E. (1977) Human cells required for macrophage migration inhibitory factor (MIF) production. *Clin. Immunol. Immunopath.* **8**, 396.

VALDIMARSSON, H., MOSS, P.D., HOLT, P.J.L. & HOBBS, J.R. Treatment of chronic mucocutaneous candidiasis with leucocytes from HL-A compatible sibling. *Lancet*, i, 469.

VALDIMARSSON, H., YAMAMURA, M., HIGGS, J.M., WELLS, HOBBS, J.R. & HOLT, P.J.L. (1973) Immune abnormalities associated with chronic muco-cutaneous candidiasis. *Cell Immunol.* **6**, 348.