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Research Article

A specific inhibitory activity of in vitro proliferative responses of normal human lymphocytes to Candida metabolic antigen was found in the serum of 6 out of 23 children with chronic mucocutaneous candidiasis. In each of the six patients, the presence of an inhibitory activity was associated with Candida-specific cellular defects, characterized by a negative-skin test and a lack of in vitro lymphocyte proliferation. The presence of a circulating inhibitor was detected during relapses of the disease and disappeared under antifungal therapy. This inhibitory effect was not associated with any toxicity on tested lymphocytes. The factor was shown to be nondialysable, thermostable, nonprecipitable with ammonium sulfate and absorbable on anti-Candida antibodies or concanavalin A-coupled agarose columns. Altogether, these results suggest that the inhibitory factor is not an immunoglobulin, but rather a polysaccharidic antigen of Candida albicans. An inhibition of Candida-induced proliferative response of normal human lymphocytes was also obtained by addition of polysacharide antigens or purified mannans from C. albicans to cultures. Candida polysaccharidic antigens appeared, therefore, to be involved in specific depression of cellular functions observed in chronic candidiasis.

Specific Inhibition of In Vitro *Candida*-Induced Lymphocyte Proliferation by Polysaccharidic Antigens Present in the Serum of Patients with Chronic Mucocutaneous Candidiasis

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ABSTRACT A specific inhibitory activity of in vitro proliferative responses of normal human lymphocytes to *Candida* metabolic antigen was found in the serum of 6 out of 23 children with chronic mucocutaneous candidiasis. In each of the six patients, the presence of an inhibitory activity was associated with *Candida*-specific cellular defects, characterized by a negative-skin test and a lack of in vitro lymphocyte proliferation. The presence of a circulating inhibitor was detected during relapses of the disease and disappeared under antifungal therapy. This inhibitory effect was not associated with any toxicity on tested lymphocytes. The factor was shown to be nondialysable, thermostable, nonprecipitable with ammonium sulfate and absorbable on anti-*Candida* antibodies or concanavalin A-coupled agarose columns. Altogether, these results suggest that the inhibitory factor is not an immunoglobulin, but rather a polysaccharidic antigen of *Candida albicans*. An inhibition of *Candida*-induced proliferative response of normal human lymphocytes was also obtained by addition of polysacharide antigens or purified mannans from *C. albicans* to cultures. *Candida* polysaccharidic antigens appeared, therefore, to be involved in specific depression of cellular functions observed in chronic candidiasis.

INTRODUCTION

Chronic mucocutaneous candidiasis (CMCC)[1] is a rare disease often revealed in the first years of life, which exhibits a high familial frequency (1). Immunological disorders observed in CMCC are mainly represented by a defect of cell-mediated immunity, generally restricted to some antigens or exclusively to *Candida albicans* (CA) itself. Negative-delayed hypersensitivity and sometimes abnormal in vitro proliferative response to *Candida* antigens were reported contrasting with normal or increased serum anti-*Candida* antibody (2–5). In some patients, the restoration of cellular responses to CA paralleled a clinical improvement with antifungal therapy, suggesting that cellular abnormalities were not a result of intrinsic lymphocytic defect (6, 7). An inhibitory activity of normal lymphocyte proliferation was described in sera from 19 CMCC patients, all of them presenting an immune cellular defect (2, 6, 8–12). In 13 cases the specificity of this inhibition seemed to be restricted to CA-induced proliferation (2, 9, 11). Because the nature of the inhibitor present in the CMCC patients sera had not been studied, the purpose of this work was to characterize physicochemical properties of the inhibitory material and to establish whether there was any correlation between presence of a detectable serum inhibitor and immunological disorders.

METHODS

Patients. 23 patients (1- to 20-yr-old) with CMCC, were studied. Anti-*Candida* antibodies were detected by electrosyneresis (13). Delayed skin reactivity was tested with candidin (1:100,000 U in 0.1 ml saline) and tuberculin purified protein derivative (PPD) (Institut Pasteur, Paris) (10 U in 0.1 ml saline). Proliferative responses to *Candida* metabolic antigen (CMA), PPD, and mitogens were tested. Table I shows

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[1] *Abbreviations used in this paper*: α-MM, alphamethylmannoside; AEF, absorbed and eluted fraction; BSA, bovine serum albumin; CA, *Candida* albicans; CMA, *Candida* metabolic antigen; CMCC, chronic mucocutaneous candidiasis; con A, concanavalin A; CSA, *Candida* somatic antigen; 3H-T, [^{3}H]thymidine; NAF, nonabsorbed fraction; PPD, purified protein derivative.

TABLE I

Responsiveness to CA, Other Antigens: PPD and Streptokinase-Streptodornase, and Mitogens of 23 Patients with Chronic Mucocutaneous Candidiasis Tested

Patients	Proliferative inhibitory effect	Skin reactivity		Proliferative response to			Antibodies to Candida Albicans
		Candidin	Other antigens*	CMA‡	Other antigens*	Mitogens§	
D.D.	+	−	+	−	+	+	+
D.H.	+	−	+	−	+	+	+
M.P.	+	−	+	−	ND	+	−
R.V.	+	−	ND	−	ND	+	+
H.G.	+	−	+	−	+	+	+
F.B.	+	−	ND	−	ND	+	+
P.J.P.	−	−	+	−	ND	+	+
T.F.	−	−	−	−	−	+	+
G.P.	−	−	+	−	ND	+	+
D.V.A.	−	−	ND	−	ND	+	+
C.S.	−	−	−	−	−	+	+
P.A.	−	−	+	−	+	+	+
T.O.J.	−	−	+	−	+	+	ND
T.O.F.	−	−	−	+	+	+	ND
T.O.M.	−	−	−	+	+	+	ND
L.C.	−	−	ND	+	NS	+	ND
L.B.	−	−	+	+	+	+	−
S.D.	−	+	+	+	+	+	−
L.S.	−	+	+	+	+	+	+
N.G.	−	+	ND	+	+	+	+
N.G.G.	−	+	ND	+	+	+	−
N.G.C.	−	+	ND	+	+	+	+
N.G.A.	−	+	ND	+	+	+	+

ND, not done.

* Skin tests with PPD and(or) streptokinase-streptodornase.

‡ Proliferative response of patient's leucocytes in the presence of CMA and homologous normal serum.

§ Phytohemagglutinin, concanavalin, and pokeweed mitogens.

the responsiveness to *Candida* antigens, other antigens or mitogens of the 23 patients. All had normal serum immunoglobulin levels and markers of thymus-derived (T) and bone marrow-derived (B) cells. Patients sera were stored at −20°C and decomplemented (56°C, 30 min) before the study.

CA antigens. CMA and *Candida* somatic antigen (CSA) were purchased from Institut Pasteur. CMA was a filtrate of CA cultures, extensively dialysed, concentrated, and lyophylized. It contained 1.5% azote and 7–10% carbohydrate (mannan, glucan, and xylane). CMA was used in all proliferation studies at a final concentration of 0.25 mg/ml except when otherwise stated. CSA was pulverized suspension of continuously agitated CA culture at 30°C in a liquid Sabouraud culture medium, dialyzed, concentrated, and lyophylized. It contained 4.7% azote and 9% carbohydrate.

Purified carbohydrate used in culture. Mannan from CA (A and B) (a gift from Dr. H. F. Hasenclever, National Institutes of Health, Hamilton, Mont. [14]), dextran (mol wt = 70,000) (Pharmacial Fine Chemicals, Inc., Uppsala, Sweden, and alphamethylmannoside (α-MM) (Sigma Chemical Co., St. Louis, Mo.) were used at a concentration of 5–2,000 μg/ml.

Fractionation of patient's sera (Fig. 1). Sera were precipitated by adding a saturated $(NH_4)_2SO_4$ (pH 7.0) solution at a final concentration of 45%. Supernates and precipitates were separated.

Precipitates were washed three times in 45% $(NH_4)_2SO_4$, dissolved in distilled water, successively dialyzed against water and acetate buffer (pH. 5.0) then passed on a DEAE-Sephadex A-50 (Pharmacia Fine Chemicals, Inc.) column equilibrated with an acetate-buffered saline (pH 5.0). Immunoglobulin (Ig)G-containing eluate was recuperated from the column by washing with the same buffer and passed on Sepharose 4B beads (Pharmacia Fine Chemicals, Inc.) covalently (BrCN) (15) bound to CMA antigens (8 mg protein/1 ml Sepharose). Column was equilibrated successively with glycine (0.5 M), phosphate (0.1 M), pH 7.0, then Tris-HCl (0.1 M), NaCL (0.1 M) buffer, pH 7.3, then washed with glycine buffer (0.1 M, pH 2.5), and finally equilibrated in Tris-HCl (0.1 M), NaCL (0.1 M) buffer (pH 7.3). IgG-containing fractions were applied on the CMA column for 2 h at room temperature. Nonabsorbed fractions (NAF) were first recuperated by washing (20-columns volumes) with Tris-HCl buffer (pH 7.3). Absorbed and eluted fractions (AEF) were obtained by washing with glycine buffer (0.1 M, pH 2.5) immediately reconstituted to pH 7.0 by addition of $\bar{K}_2H\,PO_4$ (1 M).

Supernates were fractioned by affinity chromatography on Sepharose 4B beads columns. Sepharose was bound to either bovine serum albumin (BSA) (Sigma Chemical Co.), hyperimmune anti-CMA sera raised in goat (a gift from Dr. Drouhet, Institut Pasteur), or concanavalin (con A). Columns were

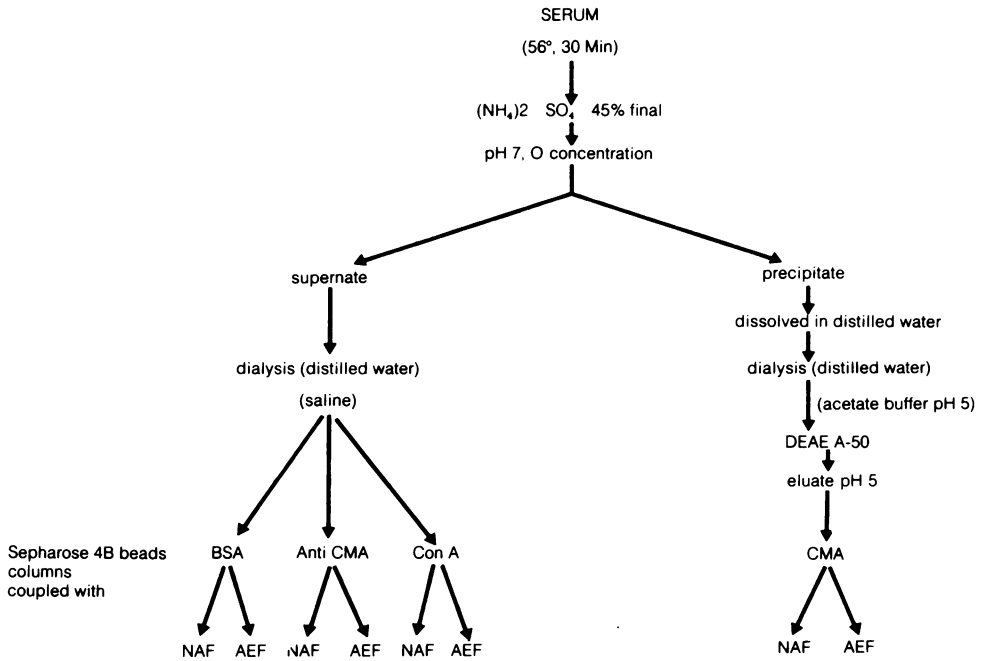

FIGURE 1 Schema of serum fractionations. Supernates and precipitates by $(NH_4)_2SO_4$, eluates from DEAE-Sephadex A-50 columns at pH 5, NAF and AEF were dialyzed against saline, concentrated by dialysis against polyethylene glycol, then dialyzed against saline and finally against culture medium before being tested.

equilibrated as indicated above. Sepharose-con A columns were then washed with α-MM (250 mM). Anti-CMA and BSA columns were washed with glycine buffer (0.1 M, pH 2.5). All columns were finally equilibrated in Tris-HCl buffer (pH 7.3). Supernatant fractions were applied on anti-CMA, con A, or BSA columns, for 2 h at room temperature. NAF were recuperated by washing (20-columns vol) with Tris-HCl buffer (pH 7.3). AEF were obtained from anti-CMA and BSA columns by washing with glycine buffer (0.1 M, pH 2.5), then immediately reconstituted to pH 7 by addition of $K_2H\ PO_4$ (1 M). AEF from con A columns were obtained by washing with α-MM (250 MM). All fractions were extensively dialyzed against NaCl (0.15 M) solutions. Disappearance of α-MM in dialysis fluids was verified by determination of carbohydrate contents.

Supernates, precipitate, and their fractions were extensively dialyzed against NaCl solutions (0.15 M) concentrated and dialyzed successively against polyethylene glycol, saline, and culture medium before assay in culture. Fractions were reconstituted to the initial volume of serum.

Fractionation of Candida antigens. The same procedures were followed for CMA and CSA which were passed on con A columns and fractionated into AEF and NAF. Estimation of proteins and carbohydrates contents was performed in serum fractions or dialyzed fluids with the Lowry et al. (16) and phenylsulphuric acid (17) methods.

Cell preparations. Normal mononuclear leukocytes from healthy volunteers with known prior sensitization to *Candida* were prepared with a Ficoll-Hypaque gradient (Pharmacia Fine Chemicals, Inc.) (18) washed three times in TC-199 medium (Gibco, Glasgow, Scotland) and cultured at a concentration of 1×10^6 cells/ml (0.5 ml/culture tube) in RPMI 1640 (Gibco) supplemented with antibiotics (penicillin G 50 U/ml, streptomycin 50 μg/ml, gentamycin 50 μg/ml, amphotericin B 0.25 μg/ml, glutamine (1.5 mM/liter) (Institut Mérieux, Lyon, France), and serum at various concentrations as indicated below.

Lymphocyte proliferation studies. Cell cultures were incubated at 37°C in a 5% CO_2 atmosphere. Cells stimulated with con A (Miles, Yeda, Israel) at a final concentration of 10 μg/ml were cultured for 4 days; cells stimulated with antigens for 6 days. 1 μCi of [^{3}H]thymidine (3H-T) (Commissariat a l'Energie Atomique, Saclay, France) per milliliter of culture fluid was added to each culture 18 h before harvesting. Incorporation of 3H-T was measured with a paper disk technique (19). Tubes were prepared in duplicate for experimental and, at least, in triplicate for control cultures, and two samples from each tube were tested. The range of normal response to CMA was 25,000–100,000 cpm/tube and spontaneous 3H-T incorporation ranged 700–1,500 cpm/tube. No attention was given to the presence of antibodies directed against blood group substances in serum samples. Indeed, preliminary experiments had established that proliferative responses in cultures performed in the presence of sera from various A, B, or O normal individuals were similar.

Assay for inhibition of Candida-induced proliferation and quantitation of the inhibitory effects. Inhibitory effect of patient's serum was studied on lymphocytes from normal *Candida*-sensitized individuals stimulated with CMA (0.25 mg/ml). Cultures were performed, with one exception, at a constant final concentration of AB^+ serum (10%) and variable concentration (0–10%) of patient's serum or their fractions. The amount of serum or serum fraction added to cultures was expressed as the percentage: volume of serum or serum fraction × 100/vol of serum or serum fraction plus volume of (AB^+) serum.

Results were compared to those obtained in control cultures (10% AB^+ serum alone). The inhibition of proliferative re-

sponses by the patient's serum or their fractions was expressed as the ratio: 3H-T incorporation (counts per minute per tube) in control culture minus 3H-T incorporation (counts per minute per tube) in experimental culture/3H-T incorporation incorporation (counts per minute per tube) in control culture ×100. Percentage of blasts among culture cells was determined on smears stained with May-Grunwald-Giemsa.

RESULTS

Inhibitory activity of unfractionated patients' sera on CMA-induced lymphocyte proliferation. The detection of an inhibitory effect present in the serum of patients with CMCC was first assayed by culturing normal lymphocytes in the presence of an optimal concentration of CMA (0.25 mg/ml) with an equal volume of AB⁺ (10%) and patient (10%) serum. Results were compared with cultures in 10 and 20% AB⁺ serum. Among the 23 patients' sera tested, 6 showed a concomitant inhibitory activity on proliferation (>50% inhibition) and blastogenesis whereas 10 normal sera tested under the same conditions did not display any of these effects (Fig. 2). For the purpose of this study, we investigated only the six sera showing an inhibitory activity of >50% inhibition.

We used suboptimal doses of antigen: CMA in stimulating normal lymphocytes in presence of suppressive material from one CMCC patient displaying an inhibitory activity (patient F.B.). We found no change in the intensity of inhibition: ≅50% from 0.025 mg to 0.50 mg of CMA. As shown in Fig. 3A, sera of four patients exhibited a strong, reproducible inhibitory activity which was directly dependent on serum concentration.

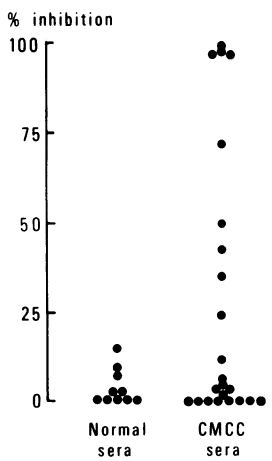

FIGURE 2 Effect of serum from 23 CMCC patients and 10 normal individuals on proliferative response of normal, *Candida*-sensitized lymphocytes to 0.25 mg/ml of CMA. CMCC serum (final 10%) was added to normal AB⁺ serum (final 10%); control cultures were performed with 10% AB⁺ serum. No difference was noted with 20% of AB⁺ serum in control cultures (data not shown).

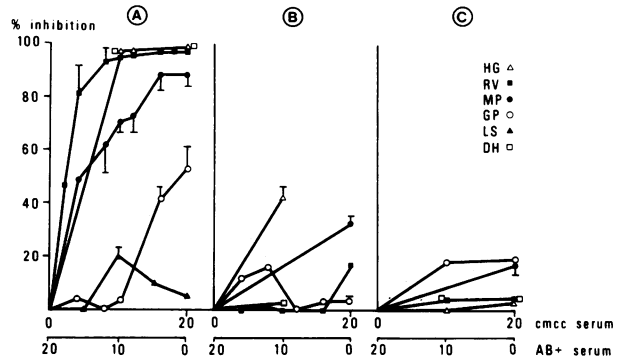

FIGURE 3 Percentage of inhibition induced by various concentrations of unfractionated patients' sera (H.G., R.V., M.P., D.H.) with or without (G.P., L.S.) inhibitory effect on proliferative responses of normal lymphocytes to CMA (A), PPD (B) and con A (C). In all experiments, the final concentration of serum was 20%. The increased concentrations of CMCC sera (0–20%) were compensated by a reciprocal decrease of normal AB⁺ serum concentration (20–0%). Results are given as the mean (±1 SD) of the percentage of inhibition in two experiments with lymphocytes of different, normal individuals.

Antigen specificity of the inhibitory activity (Fig. 3). To establish whether the inhibitory activity observed was specific for CMA-induced proliferation, the effect of patients' sera was simultaneously studied on PPD- or con A-induced lymphocyte proliferative responses. Only one patient's serum (H.G.) displayed a mild inhibitory activity on PPD-induced lymphocyte proliferation. This effect was significantly lower than that obtained with the same serum on CMA-induced proliferation ($P < 0.01$) (Student's t test).

Absence of cytotoxicity of patients' sera on normal lymphocyte and reversibility of the inhibitory effect. The viability (trypan blue exclusion) of normal lymphocytes, 6 days after stimulation with CMA, was identi-

TABLE II
Percentqge of Inhibition Induced by Unfractionated Patients' Sera or Their Fractions on Proliferative Responses of Normal Lymphocytes to CMA.

	Unfractionated sera	Supernatant fractions	Precipitated fractions
	%	%	%
Pooled AB⁺ serum	0	0	11.5
G.P.	3.7	0.2	0
H.G.	97.3	96.5	32.8
D.H.	98.6	97.0	18.7
D.D.	98.3	95.8	23.5
R.V.	96.7	96.4	0
F.B.	50.0	94.9	31.0

Inhibitory patients' sera (H.G., D.H., D.D., R.V., F.B.), one noninhibitory patient's serum (G.P.), supernatant fractions and ammonium sulfate precipitated fractions were studied.

cal whether cultures were performed in the presence of normal AB+ serum (10%) (viability: 77±5%) or in the presence of inhibitory sera (10%) added to 10% AB+ serum (viability: 74±10%). The spontaneous incorporation of 3H-T by normal, nonstimulated lymphocytes cultured for 6 days was the same whether normal serum (1,230±165 cpm) or inhibitory serum plus normal serum (vol/vol) (1,159±120 cpm) were added to the cultures.

Normal leukocytes were incubated for 24 h in the presence of inhibitory or normal serum, then washed and stimulated by CMA in normal serum for the remaining 5 days, showed identical capacity of proliferation.

Thermostability and dialysis studies. When frozen at −20°C for periods varying from 1 day to 2 yr, patients' sera maintained their inhibitory activity. The effect was not altered by dialysis or by heating at 56°C for 30 min. In other experiments, heating at 80°C for 30 min followed by a centrifugation (3,000 rpm for 10 min) to remove denatured proteins, did not lessen the inhibitory effect. Control sera did not exert any inhibitory activity after heating at 80°C for 30 min.

Inhibitory activity of serum fractions obtained by salt precipitation on CMA-induced lymphocyte proliferation. When sera were fractionated by precipitation in ammonium sulfate, the precipitable fraction did not retain the same inhibitory activity that was recovered in the supernates. The same fractions from either a pool of normal sera or one CMCC patient's serum without inhibitory activity, did not exhibit any inhibition (Table II). No inhibitory activity, initially present in patients' sera was recovered in IgG-containing eluates obtained from a DEAE-Sephadex A-50 column, or in IgG eluated from a Sepharose CMA column.

Inhibitory activity of serum fractions obtained by affinity chromatography on CMA-induced lymphocyte proliferation (Fig. 4). Supernatant fractions obtained after ammonium sulfate precipitation from three inhibitory patients' sera were passed through anti-

Adsorbent column		Sepharose-BSA control	Sepharose-anticandida goat antiserum		Sepharose-concanavaline A	
Nature of serum		FB	FB	HG	FB	DD
Protein concentration in µg/ml	S fraction	7000	7000	4550	7000	ND
	Non adsorbed fraction	5530	4575	ND	3800	4050
	Adsorbed and eluted fraction	< 50	105	< 50	250	< 50
Carbohydrate concentration in µg/ml	S fraction	490	490	400	490	280
	Non adsorbed fraction	370	300	260	150	70
	Adsorbed and eluted fraction	< 20	125	< 20	205	120

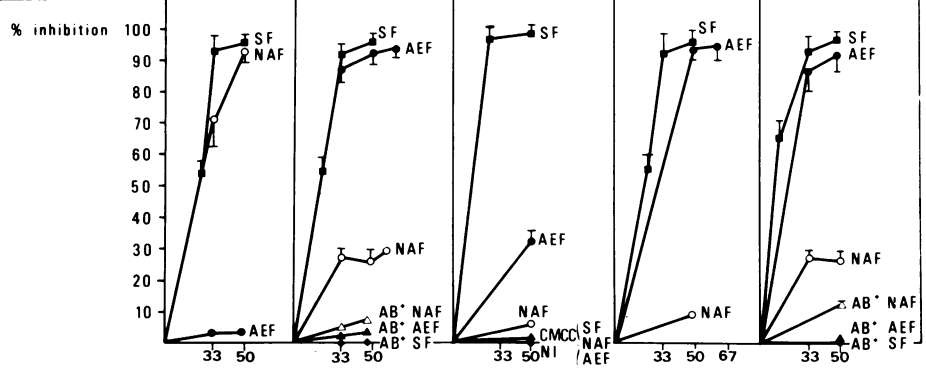

FIGURE 4 Percentage of inhibition induced by ammonium sulfate supernates (SF) and their fractions on proliferative responses of normal lymphocytes to CMA. Ammonium sulfate supernates were passed through anti-*Candida* antiserum, con A- or BSA-Sepharose columns. Figures indicate the percentage of inhibition observed when SF, NAF and AEF were added to cultures. The amount of serum fractions added to cultures was expressed as the percentage of volume of serum fraction ×100/volume of serum fraction + volume of (AB+) serum. This ratio is indicated in absciss. Figure illustrating F.B. and D.D. sera, also indicate the effect of SF, NAF, and AEF fractions of a normal serum (AB+) and the figure illustrating HG serum includes results obtained with SF, NAF, and AEF fractions from a noninhibitory CMCC serum (CMCC NI) (patient G.P.).

Candida antiserum, con A- or BSA-Sepharose columns. The same fractions from normal AB^+ and noninhibitory patients' sera were studied. The inhibitory activity present in supernatant fractions was not detected in NAF from anti-*Candida* antiserum column. In contrast the inhibitory activity was found in AEF of anti-*Candida* column. When compared with supernatant fraction, AEF had a higher carbohydrate protein ratio. The inhibitory activity was also retained on con A-Sepharose columns and was eluted using α-MM. Again, AEF from con A column was enriched in carbohydrates when compared with the supernate. In contrast, BSA columns did not retain the inhibitory activity. When normal sera or noninhibitory patient's serum were passed through anti-*Candida* or con A columns, no inhibitory activity was retained.

Effect of CMA, CSA, or their carbohydrate-rich components on CMA-induced lymphocyte proliferation (Fig. 5). In these experiments, CMA and CSA were passed through a con A-Sepharose column. The inhibitory activity of NAF and AEF were studied at various concentrations of carbohydrate contents. AEF, that contained carbohydrate with affinity to con A appeared to exert, at the same carbohydrate concentration, a stronger inhibitory activity than NAF or crude (CMA or CSA) antigens.

Inhibitory activity of mannan from CA or CMA-induced lymphocyte proliferation (Fig. 6). An inhibitory activity was observed when mannan from CA (A or B) was added at a concentration of 50 μg/ml or more to the culture. This inhibitory effect appeared to be specific for CMA because concentrations ranging from 50 to 500 μg/ml did not modify PPD- or con A-induced proliferation. However, a mild inhibition (50%) was detectable on PPD-induced proliferative response when a much larger concentration (1,000 μg/ml) of mannan was used. Mannan added to non-stimulated lymphocyte cultures did not exert any mitogenic activity whatever the concentration used (5–2,000 μg/ml). Dextran and α-MM did not exhibit any inhibitory activity on CMA-induced lymphocyte proliferation.

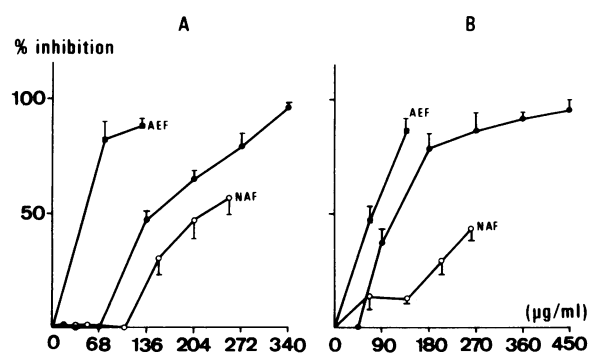

FIGURE 5 Percentage of inhibition induced by CMA, CSA, or their fractions on the proliferative response of normal lymphocytes to CMA (0.25 mg/ml or 17 μg/ml carbohydrate). CMA and CSA were passed on con A-Sepharose columns. Figures indicate the percentage of inhibition observed with crude antigen (●) (CMA = A, CSA = B), NAF (○), or AEF (■). The abscissa show the carbohydrate contents (μg/ml) of the preparations tested.

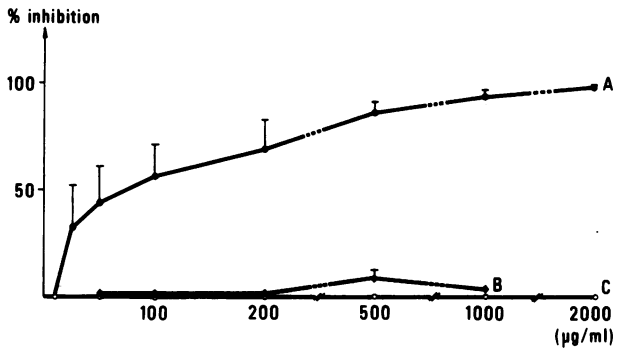

FIGURE 6 Percentage of inhibition induced by mannan from CA (A), dextran (B), and α-MM (C) on proliferative responses of normal lymphocytes to CMA. Carbohydrate concentrations varied from 5 to 2,000 μg/ml of culture.

Longitudinal studies of clinical and immunological data of patients with serum inhibitor (Table III). In three patients under antifungal therapy, clinical improvement was observed together with the disappearance of the inhibitory activity when proliferative response and skin reactivity to CMA became present.

DISCUSSION

An inhibitory activity on *Candida* antigens-induced proliferative response of normal lymphocytes was detected in 6 out of 23 patients' sera, having chronic mucocutaneous candidiasis. This inhibitory activity appeared to be directly dependent on a patient's serum concentration, allowing for its quantization not achieved in previous studies (2, 6, 8, 10, 11). These previous observations have shown that specific inhibition for *Candida*-induced proliferation was found in most (13 out of 19) CMCC patients' sera tested (2, 9, 11). In six inhibitory patients' sera investigated in this study, only one was found to be slightly inhibitory on PPD-induced proliferative response. As already described in another series of CMCC patients (2), the six patients who had a serum inhibitory activity exhibited a selective unresponsiveness to *Candida* antigens, i.e., negative-delayed skin hypersensitivity and absence of in vitro proliferative response in homologous serum.

Our study was aimed at further characterizing the nature of the inhibitor present in the serum of the six patients. Because an inhibition was observed at a 2% final concentration of patient's sera, the effect

TABLE III

Clinical and Immunological Follow-up of Patients D.H., H.G., and M.P. and the Presence of a Detectable Serum Inhibitor

Patient	Date	Candidiasis manifestations		Percentage of inhibition of normal lymphocyte proliferation induced by CMA	Mitogenic index*	Skin-delayed hypersensitivity to CMA
		Cutaneous	Digestive			
D.H.	2/74	±	+	98.5	1.0	−
	5/76	−	−	0	32.6	+
	1/77	−	+	73.0	ND	ND
	2/77	−	±	27.0	10.0	+
H.G.	2/74	+	+	97.3	5.0	−
	2/76	−	−	0	48.0	+
M.P.	3/71	+	+	70.5	1.0	−
	9/72	−	−	0	44.0	+

* Mitogenic index: 3 H-T incorporation (in cpm) in CMA-stimulated cultures/3 H-T incorporation (in cpm) in nonstimulated control cultures.

appeared to be an active, but not cytotoxic phenomenon, and not because of a lack of proliferation supporting substances. Because antibodies against *Candida* antigens are generally found at a high titer in CMCC (1, 3, 20, 21) it was previously suggested that either antibodies or immune complexes might be involved in the inhibitory activity (2, 5, 8). Our study, however, indicates that this effect was not found in IgG-containing fractions, but was recovered in the supernates of ammonium sulfate-treated sera. The role of immune complexes or antibodies in inhibiting in vitro cell-mediated immunity to CA in CMCC is thus unlikely. Furthermore, one of the six inhibitory patients' sera (M.P.) did not contain a detectable antibody directed against *Candida* antigens. Our observation that the inhibitory activity was retained on anti-*Candida* antibody-coated columns and recovered in eluted fractions strongly suggests that the inhibitor is *Candida* antigen(s) itself. Thus, it could be argued that inhibition of *Candida* antigens-induced proliferative response was caused by a shift in the concentration of *Candida* antigen required to elicit maximal proliferative response. This, however, could be ruled out because the inhibitory effect of patient's sera was observed over a broad range of CMA concentrations, including suboptimal doses.

Evidence, for the polysaccharidic nature of the inhibitory substance(s) was deduced from the following observations. First, inhibitory fractions eluted from anti-*Candida* antibodies columns were enriched in carbohydrate contents. Second, the inhibitor was shown to be thermostable at 80°C. Third, the inhibitory activity could be retained and eluted from con A-coated columns, indicating that mannose and (or) glucose residues are structural components of the inhibitor. Because mannan and glucan are quantitatively the two main polysaccharidic components of the *Candida* cell wall (22–25) we looked for a possible inhibition induced by fractions of *Candida* antigens enriched in those carbohydrates and particularly by their fractions absorbed on con A-coupled columns. Indeed, mannose and (or) glucose residues-enriched materials from CMA and CSA displayed, at low concentration, a significant inhibition of *Candida*-induced lymphocyte proliferation. Furthermore, purified mannan (extracted from *Candida*) showed a comparable specific inhibition on *Candida*-induced proliferation. Our results, therefore, suggest that *Candida* antigens and possibly mannan, circulate in serum of patients with CMCC and are responsible for the observed specific inhibitory activity. However, the possibility that other polysaccharidic parts of the *Candida* organism, including glucan, participate in the inhibitory activity has not been ruled out.

It must be considered whether there is a link between presence of a serum inhibitor, cellular unresponsiveness to *Candida* antigens, and the chronic course of the infection. Longitudinal observations in three patients (Table III) showed a correlation between clinical improvement under antifungal therapy, disappearance of the serum inhibitory activity, and appearance of in vivo and in vitro cell-mediated responses to *Candida* antigens. The reversible specific cellular deficiency observed in CMCC could thus be secondary to an overload of *Candida* polysaccharide antigens which may contribute to the chronicity of the disease by inducing the immune cellular unresponsiveness. Indeed, free *Candida* antigens mannan, were detected in the serum of patients with acute invasive candidiasis (26). High antibody titers against *Candida* antigens (including antimannan antibodies) (21) are usually detected in CMCC patients (4). This,

however, does not exclude that poorly immunogenic *Candida* antigens, including glucan (27), may escape the humoral response. In experiments with anti-*Candida* antibody coupled columns, glucan could be absorbed as the peptido-gluco-mannan complex of the *Candida* cell wall (24). A more attractice hypothesis, however, could be an impaired catabolism of polysaccharidic materials by macrophages of patients with CMCC. In that respect, the physiological resistance of glucan (which provokes granuloma when injected intravenously in mice) to enzymatic degradation by macrophages should be underlined (28). We may speculate that an abnormality of the macrophages in patients with CMCC may underlie the disease. This situation is, somehow, reminiscent of high-responder mouse strain in which high production of antibodies contrasting with a low catabolism of antigens by macrophages has been demonstrated (29).

The mechanism of the inhibition of free antigens of the T-cell proliferation remains a matter of speculation. One could envisage a competition for specific antigen receptors on the membrane of T cells, circulating antigen, and antigens handled by macrophages (30). The antigenic excess may also induce a specific suppressive cell active on the cellular but not humoral immune response.

Some CMCC patients with a reversible specific cellular immune defect, that we have observed or who have been reported (31), had no detectable serum inhibitor. It is possible that other mechanisms are involved in the acquired immunodeficiency of those patients (1, 5, 31). In these patients, it cannot be ruled out that a local, nondiffusible, inhibitory activity of *Candida*-specific cellular response is mediated by high concentration of *Candida* polysaccharides in the skin and mucosal lesions. In all cases, clearing the overload of *Candida* antigens by potent and prolonged antifungal therapy might be the best way to restore the cellular immune response.

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