Proliferation of human peripheral blood mononuclear cells induced by *Candida albicans* and its cell wall fractions

CLARA M. AUSIELLO,*† G. C. SPAGNOLI,*† MARIA BOCCANERA,† IDA CASALINUOVO,†
F. MALAVASI,‡ C. U. CASCIANI,* and A. CASSONE†§

* Istituto CNR di Tipizzazione Tissutale e Problemi della Dialisi, L'Aquila,

† Laboratorio di Batteriologia e Micologia Medica, Istituto Superiore di Sanità, Rome, and

‡ Istituto di Genetica Medica, Università di Torino, Italy

Summary. Glutaraldehyde-inactivated cells and cell-wall fractions of *Candida albicans* were studied for their capacity to induce or inhibit the in-vitro proliferation of human peripheral blood mononuclear cells (PBMC), as measured by ³H-thymidine incorporation. Both the intact cells (CA) and a phosphorylated gluco-mannan-protein complex of the cell wall (GMP), in µg doses, were strong inducers of PBMC proliferation, with a peak of activity at 6–9 days of culture and varying with the PBMC donor. A significant but much lower proliferation was observed on exposure of PBMC to a low-protein (<3% by weight) mannan component (M) of the cell wall. Both a hot-alkali extracted mannan-protein complex (M-alk), comparable to GMP in crude chemical composition, and an alkali-insoluble cell-wall glucan (GG) were inactive. None of the *Candida* fractions induced a lymphoproliferation of umbilical cord blood cells and all fractions, except GG, were equally effective in binding human anti-*Candida* antibodies as shown by a sensitive ELISA-inhibition assay. Moreover, a monoclonal antibody against the class II determinant of the HLA complex inhibited PBMC proliferation irrespective of the *Candida* antigen used. Taken together, the data shows that in inducing lymphoproliferation, *Candida* fractions act as specific antigens rather than as non-specific mitogens. Use of intact *Candida* cells and chemically-defined cell-wall components appears preferable to use of undefined antigenic mixtures as stimulators of PBMC proliferation.

Introduction

Most healthy individuals possess significant levels of antibodies against the immunodominant mannan chains of the outer cell wall of *Candida albicans* (Odds, 1979). Lymphocyte proliferation in response to *Candida* extracts, mixtures of undefined antigenic components, are currently used to monitor T-cell dependent immune responses (Brunham *et al.*, 1983; Cox, 1983).

The ability of *Candida* preparations to stimulate lymphocyte blastogenesis or specific antibody synthesis or both *in vitro* has been reported by Piccollella *et al.* (1980), Gettner and MacKenzie (1981), Durandy *et al.* (1983), De Repentigny and Reiss (1984) and Wirz *et al.* (1984). However, either

crude antigenic mixtures were used, or no details of the chemical composition and antigenicity of the stimulant fraction were given. Moreover, specific antigenic activation was seldom separated from possible non-specific mitogenic effects. In this study, we report the capacity of purified, chemically-defined mannan-rich components of the cell wall of *C. albicans* to modulate human lymphocyte proliferation *in vitro*. Because lymphocyte proliferation *in vivo* during colonisation or infection by *Candida* reasonably involves intact cells of the micro-organism as well as the released antigens, we have also compared the proliferative responses elicited by whole microbial cells to those obtained with cell-wall components.

Materials and methods

Organism and growth conditions

C. albicans, strain BP serotype A, from the collection of the Istituto Superiore di Sanità, Rome, Italy, was used

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§ Correspondence and requests for offprints should be sent to: Dr A. Cassone, Medical Mycology Unit, Laboratory of Bacteriology and Medical Mycology, Istituto Superiore di Sanità, Viale Regina Elena, 299–00161 Rome, Italy.

throughout. This strain, identified as described by Lodder (1970), was routinely maintained on Sabouraud-dextrose agar at 28°C and, for experimental use, grown with mild agitation in liquid Winge medium (glucose 0.2% w/v, yeast extract (BBL, Cockeysville, MD, USA) 0.3%) until stationary-phase growth (48 h) was established. Dense washed-cell suspensions were inactivated with glutaraldehyde (Polysciences, PA, USA) 2% v/v in phosphate buffer (0.12 M, pH 7.2) for 1 h at 37°C, then washed repeatedly with pyrogen-free distilled water for use as whole cells (CA). Yeast-mycelial conversion was induced by suspending 2×10^7 cells/ml in N-acetylglucosamine medium as reported by Mattia and Cassone (1979). Mycelial growth was stopped after incubation for 270 min at 37°C, and hyphal elements (>95%) inactivated and treated as above.

Preparation of CA-antigenic fractions

A cell-wall fraction (GMP) was obtained by autoclaving washed yeast cells and precipitating the supernate in cold ethanol, according to Sutherland and Wilkinson (1971). After deproteination by chloroform-butanol (Sutherland and Wilkinson, 1971), the aqueous phase was extensively dialysed against H₂O and then lyophilised. The yield ranged from 0.2 to 0.5% of cell dry weight, and the material consisted essentially of a glucomannan-protein complex (see below). For ELISA-inhibition experiments, a similar fraction was prepared from *Saccharomyces cerevisiae* as a control. A mannan fraction (M) was prepared as described by Peat *et al.* (1961). M-alk, a mannan-rich preparation was obtained by hot-alkali extraction of acid-treated cell walls, as reported by Korn and Northcote (1960). An insoluble glucan fraction in the form of "glucan ghosts" (GG) was prepared by cycles of acid and alkali extraction at 100°C as described by Cassone *et al.* (1978). This fraction contained about 3% chitin (Cassone *et al.*, 1981).

Cell separation

Blood samples were obtained from healthy adult volunteers in preservative-free heparin (Eparin Vitrum, Como, Italy) 100 IU/ml. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a density gradient (Lymphoprep, Nyegard, Oslo, Norway) according to Böyum (1968) and washed twice in RPMI medium (Gibco, Grand Island, NY, USA). Cells were then resuspended in RPMI medium supplemented with 10% pooled AB serum and antibiotics (penicillin 100 IU/ml, streptomycin 0.1 mg/ml; Gibco) (complete medium). In some experiments, PBMC from umbilical-cord blood, separated as described above, were used as responder cells.

Proliferation assays

Cultures were set up in flat bottomed 96-microwell trays (Nunc, Denmark). PBMC (2×10^5) were stimula-

ted with CA or its fractions, at the concentrations indicated, in single experiments. All tests were performed in triplicate. Trays were incubated at 37°C in CO₂ 5% and the cultures harvested on day 6, unless otherwise stated. Radiolabelled thymidine (methyl-³H-thymidine, 25 Ci/mMole, 1 mCi/ml, Amersham, UK) was added to a final concentration of 0.5 µCi/well 18 h before harvesting. Cultures were harvested by a Titertek cell harvester (Skatron, Oslo, Norway) and ³H-thymidine incorporation measured by the two channel ratio, using the external standard method, with a Beckman (Irvine, CA, USA) LS 9100 liquid scintillator system.

In some experiments "commitment" of PBMC proliferation after stimulation with the various *C. albicans* preparations was evaluated as follows: 10^6 PBMC in 1 ml of complete medium were incubated for up to 2 h with an optimal concentration of CA, GMP and M, then extensively washed to remove free antigen and further processed as for the proliferation assay.

Mitogen-induced blastogenesis

Non-specific mitogen-induced blastogenesis was determined by culturing 2×10^5 PBMC in 0.2 ml of complete RPMI medium in flat bottomed 96-microwell trays in the presence of a 1 in 100 dilution of commercial phytohaemagglutinin PHA (HA 15; Wellcome, UK). Pulsing of cultures with ³H-thymidine and isotope-incorporation measurement were performed as described above and the incorporation was stopped on day 3 of PHA stimulation.

Inhibition of CA-induced proliferative response

In a series of experiments, 2×10^5 PBMC were incubated at 37°C for 1 h with scalar concentrations of soluble mannan fractions from 0.5 to 100 µg/ml. An optimal PBMC-proliferation-inducing amount of CA was then added and the proliferative response evaluated on day 6 as described. In other experiments, a monoclonal antibody (AA 384) against MHC Class-II antigen, prepared and characterised as described by Malavasi *et al.* (1984), was added at the time of culture seeding to a final concentration of 10 µg/ml.

Analytical determinations

Protein concentrations were determined by the method of Lowry *et al.* (1951) and carbohydrate concentrations with anthrone reagent as described by Herbert *et al.* (1971). For a combined determination of mannan and glucan, the fraction was divided into two parts: one for total carbohydrate and the other for the determination of mannan as the insoluble copper complex after addition of KOH (1.0 M final concentration) and Fehling solutions (Peat *et al.*, 1961). The glucan estimate was obtained by subtracting the mannan concentration from that for total carbohydrate. In the estimation of mannan, allowance was made for the lower absorbance (55%) that this polysaccharide gives relative to the glucose stan-

dard. Phosphorus present in mannan preparations was determined in perchloric acid-treated samples by the phosphomolybdate complex formation as reported by Herbert *et al.* (1971).

NMR measurements

^{31}P NMR spectra of *Candida* antigens were taken at 4°C by means of a Varian FTXL 100-15" spectrometer working at 40.5 MHz, interfaced to a 620-L computer under conditions of broad-band proton decoupling. The field:frequency ratio of the spectrometer was stabilised by locking on D_2O resonance. Each spectrum represented the Fourier transform of accumulated free induction decay, obtained with a sequence of 60° pulses and acquisition time of 1.50 s. The chemical shift was measured in ppm relative to the 85% w/v phosphoric acid as external standard.

Enzyme-linked immunosorbent assay (ELISA)

Assays were performed in polystyrene microtitration plates (Dynatech, Microelisa, CA, USA). Of the antigenic fractions at concentrations of 5 µg/ml, 200 µl were added to wells, held overnight at 4°C, and subsequently rinsed with phosphate-buffered saline (PBS; 0.1 M, pH 7.4) containing Tween 20 × 3 0.05% v/v. Then 200 µl of hyperimmune human serum (ELISA titre >128 000) from a candidosis-convalescent donor was added, at the desired dilution, and plates incubated for 1 h at room temperature. After careful washings with Tween 20—PBS buffer, 200 µl of a 1 in 1000 dilution of goat anti-human IgG-alkaline phosphatase conjugate (Sigma Chemical Co., St Louis, MO, USA) was added for 1 h and enzyme activity was detected by adding nitrophenyl-phosphate reagent (Sigma). The reaction was terminated by adding 50 µl of a 3 M NaOH solution after 20 min. In some experiments, ELISA-inhibition tests were performed with cell-wall preparations of *C. albicans*. Compounds tested as inhibitors were added, at suitable dilutions, to hyperimmune human serum and left overnight at room temperature, then the mixtures were added to antigen-coated wells and the immunosorbent assay performed as described above. The ELISA tests were read with the automated microreader Titertek Multiscan (Skatron, Oslo, Norway), set at 405 nm and blanked against air. Tests were performed in triplicate and the OD of the well without a *Candida* coating antigen was taken as the background reading. A positive test was one with at least twice the absorbance of the background reading and the inhibition was taken as significant only when the mean value of triplicate samples with inhibitor was at least 50% less than the mean value of triplicate samples without inhibitor, at any given serum dilution.

Statistical analysis

Statistical analysis was performed by Student's *t* test (un-paired data).

Table I. Proliferative response* to CA by human PBMC, as measured by ^3H -thymidine incorporation

Material	Dose (µg/ml)	^3H -thymidine incorporated† (dpm × 10 ⁻³ ± SD) by PMBC from		
		donor 1	donor 2	donor 3
none‡	...	4.6 ± 1.7	3.9 ± 0.2	2.2 ± 1.2
none§	...	2.1 ± 0.1	1.6 ± 0.3	0.9 ± 0.1
CA	50	52.1 ± 17.6	158.6 ± 19.2	73.8 ± 21.8
CA	5	41.1 ± 7.7	22.3 ± 2.0	79.9 ± 21.3
CA	0.5	15.6 ± 7.3	17.1 ± 6.2	7.2 ± 2.1
PHA	1 in 100 dilution	232.9 ± 41.4	261.1 ± 14.9	126.5 ± 13.2

* Proliferation was assessed in triplicate samples each containing 2×10^5 PBMC in a final volume of 0.2 ml/well.

† ^3H -thymidine was added 18 h before harvesting the culture. Thymidine incorporation values were measured on day 3 after addition of PHA or day 6 after addition of CA.

‡ § Controls for PHA and CA stimulated cultures, respectively.

|| Used at 1 in 100 dilution of a commercial preparation.

Results

Capacity of inactivated cells of *C. albicans* to stimulate human lymphocyte blastogenesis *in vitro*

The proliferative response of PBMC to CA was assessed in 25 healthy donors. Table I shows ^3H -thymidine incorporation values in a representative experiment with three donors. CA strongly induced lymphoproliferation, optimal responses being recorded in the concentration range 5–50 µg/ml; the response also depended on the specific donor. The PBMC of all donors responded to the non-specific activator PHA. CA, at concentrations up to 50 µg/ml, did not significantly affect the response to PHA ($p > 0.05$; data not shown).

The kinetics of the proliferative response induced by CA was also studied. Fig. 1 shows that, with PBMC from a single donor, the optimal proliferative response was detected on day 9 of PBMC culture; the response then declined. With PBMC from a panel of donors, the optimum time of proliferation was in the range 6–9 days. In no case was a proliferative PBMC response observed on day 3, when the PHA-induced proliferation was usually maximal.

Because *C. albicans* may grow, either *in vitro* or *in vivo*, in two different forms—yeast (Y) or mycelial (M)—and these may express form-specific antigens (Smail and Jones, 1984), we also compared the abilities of Y and M forms to stimulate blastogenesis. PBMC from all the donors responded with a similar efficiency and kinetics to the two growth forms (data not shown).

Fig. 1. Kinetics of CA-induced proliferation in a representative donor. PBMC (2×10^5) were cultured (triplicate samples) in the absence (○—○) or in the presence (●—●) of CA 50 µg/ml. ³H-thymidine incorporation was measured on the indicated days. The vertical bar indicates $\pm$ SD.

Chemical and antigenic properties of cell-wall fractions

Comparative experiments were undertaken with CA and three mannan-rich fractions with the biochemical properties shown in table II and fig. 2. GMP is a cell wall fraction characterised by: (i) relatively high protein content (8% by weight); (ii) mannan as predominant polysaccharide component; and (iii) a high phosphorus:mannose ratio, as demonstrated both by chemical determination (table III) and by spectrometric ³¹P NMR analysis (fig. 2) which showed that phosphorus was present as phosphodiester (a single peak with a chemical shift at around 1.0 ppm with respect to an inorganic phosphate standard).

Table II. Chemical composition of whole cells and cell-wall fractions of *C. albicans*

Material	Protein	Polysaccharide	Mannan	Phosphorus
CA*	27.3	23.0	8.1 (35.2)†	N.D.
GG*	<0.1	94.3	1.2 (1.27)†	N.D.
GMP‡	8.0	85.0	83.7 (98.4)†	6.4
M-alk‡	12.0	75.0	66.6 (88.8)†	N.D.
M‡	3.5	95.8	88.0 (91.8)†	6.0

N.D. = not detected.

* Values expressed as percentages of cell dry weight.

† Values expressed as percentages of lyophilised material; the yield in all cases was not lower than 95%.

‡ () = Percentage of total polysaccharide.

Fig. 2. ³¹P NMR spectrum of GMP fraction of *C. albicans*. The spectrum was taken at 40.5 MHz, 4°C and represented 15 000 free induction-decays.

The cell-wall component M was mannan with protein c.3% by weight and phosphorus 6% in phosphodiester bonds (data not shown). M-alk consisted of c. 75% polysaccharide with a predominant mannan composition and about 12% protein. Finally, insoluble glucan, as rigid cell wall skeletons ("glucan ghosts") and essentially devoid of mannan (1.2% by weight) was also examined (table II).

GMP and M, but not M-alk, acted as solid-phase antigens in the detection of human anti-*Candida* antibodies in the ELISA indirect technique. M-alk inhibited the immunosorbent assay with GMP or M as coating antigens, and its inhibitory potency was comparable to that of GMP and M, as shown in fig. 3. The specificity of this reaction was shown by the absence of inhibition with GMP or M-alk from *S. cerevisiae*.

Lymphocyte blastogenesis induced by cell-wall fractions

Stimulation of human lymphocyte blastogenesis by cell-wall fractions is shown in fig. 4, which shows data from independent experiments with PBMC from two donors. Soluble GMP was most active, with an optimal response in the dose range 50–100 µg/ml. Although to a different extent, all the donors tested were as responsive to GMP as they were to CA. M fraction was active but the proliferation was less and much more variable than with GMP. The activities of the soluble fractions

Fig. 3. Inhibition of indirect ELISA (GMP coating antigen, hyper-immune human serum as antibody) by M (—▲—), M-alk (—●—), and GMP (—■—) from *C. albicans* or GMP from *S. cerevisiae* (○—○).

and CA in the PBMC of all donors are compared in fig. 5. Induction by GMP and M fractions required contact with PBMC for only 2 h to "commit" the responder cells to maximal proliferation as observed in experiments where the PBMC were treated for various periods and then the antigens were washed away and the PBMC were reincubated up to 6 days without any stimulant (fig. 6). The M-alk and GG preparations were completely ineffective in stimulating blastogenesis at any concentration up to 100 µg/ml in the PBMC tested (fig. 4).

Because yeast mannan has been reported to suppress lymphocyte blastogenesis (Fischer *et al.*, 1978; Nelson *et al.*, 1984) we studied the capacity of soluble wall fractions of *C. albicans* to inhibit CA-induced lymphoproliferation. PBMC were incubated for 1 h at 37°C with the soluble component, then an optimal concentration of CA was added and the culture tested for ³H-thymidine incorporation, in the conditions described. As shown in table III, M and M-alk significantly inhibited CA-induced lymphoproliferation. PBMC treated with both these fractions remained viable for at least 9 days from the beginning of the treatment with no evidence of toxicity. M and M-alk did not inhibit PHA-induced proliferation.

Supplementary studies on the proliferative response of human PBMC to CA and cell-wall fractions

The experiments reported did not fully discriminate against a possible non-specific mitogenic effect of the preparations. Therefore, experiments were

performed with PBMC from umbilical-cord blood; the proliferative responses of PBMC to CA and CA-materials were inhibited by monoclonal antibodies directed against HLA-determinants. As shown in table IV, umbilical-cord blood lymphocytes, while optimally responding to PHA, were not stimulated to proliferate by CA or GMP. In other experiments, not shown, the monoclonal antibody A384, directed against a monomorphic determinant, class-II specific HLA DR molecule (Malavasi *et al.*, 1984), inhibited the proliferative response of PBMC to all the *C. albicans* preparations.

Discussion

In this study, the in-vitro proliferation of PBMC of normal, healthy individuals in response to chemically-characterised cell-wall fractions of *C. albicans* has been compared with that induced by whole *C. albicans* cells (CA). Although CA was the most powerful stimulant of PBMC proliferation, soluble mannan and glucomannan complexes were also active. The insoluble glucan and an alkali-degraded mannan-protein complex were devoid of lymphocyte stimulating activity. Mannan components, in addition to PBMC stimulatory activity, were shown to inhibit the lymphoproliferation induced by intact CA.

The soluble mannan fractions GMP and M, which induced significant lymphoproliferation, contained constituents which bind anti-*C. albicans* antibodies, as shown by the ELISA results with high-titre human serum. Moreover, both fractions specifically inhibited the haemagglutination reaction between the same serum and *C. albicans*-mannan-coated red blood cells (data not shown). This antigenicity, together with the chemical composition, suggests that both GMP and M owe their antigenicity to mannan structures and that their capacity to induce lymphoproliferation is due to antigenic rather than non-specific activation. Evidence for this conclusion came from experiments performed with PBMC from umbilical-cord blood, which showed an optimum response to PHA but no response to *C. albicans* antigens. Experiments showing the kinetics of ³H-thymidine incorporation (maximum peak at days 6–9 of the culture), the existence of a "committing" step in the proliferation, and the capacity of a monoclonal antibody directed against a monomorphic determinant of MHC class-II antigens (Malavasi *et al.*, 1984) to inhibit strongly both CA- and mannan-induced proliferation, present further evidence in support of the above conclusion.

Fig. 4. PMBC proliferation in response to cell-wall fractions of *C. albicans*. The data represent dose-response curves, measured as ^3H -thymidine incorporation on day 6—GMP, —■—; M, —▲—; M-alk, —●—; GG, —◇—. Control values of unstimulated cultures were $(10^{-3}) 3.9 \pm 0.2$ (donor 1) and 2.1 ± 3 (donor 2).

Fig. 5. PMBC proliferative responses of healthy human donors to CA $5 \mu\text{g/ml}$ (●), GMP $50 \mu\text{g/ml}$ (■), M $50 \mu\text{g/ml}$ (▲), unstimulated cultures (○). The proliferative response was assessed by ^3H -thymidine incorporation on day 6.

The GMP fraction described here was a very active, soluble antigenic stimulator which might correspond to the antigenic activator (MPPS) reported by Piccolella *et al.* (1980) although these authors reported no details of their preparation. Chemical analysis of GMP showed it to be a glucomannan protein complex with phosphorus present in the form of phosphodiester bonds, as shown by ^{31}P NMR spectra of the compound, and as expected from the nature of the immunodominant mannan chain (Suzuki and Fukazawa, 1982).

Although less effective, significant lymphoproliferation was obtained with M, a low-protein mannan preparation. This mannan was fractionated into several components by DEAE-Sephadex chromatography and it also had a prominent phosphodiester peak in ^{31}P NMR spectrum (data not shown). A failure of low-protein mannan to stimulate lymphocyte blastogenesis or specific antibody synthesis *in vitro* has been reported by Gettner and MacKenzie (1981), Durandy *et al.* (1983) and Nelson *et al.* (1984). The reasons for this are not known but mannan preparations of similar crude chemical composition derived from different strains of *C. albicans* may vary in immunogenicity. Moreover mannan may be degraded when an alkali extraction step is included during preparation, as shown here with M-alk. However, alkali-degraded

Fig. 6. Time necessary for the induction of PBMC proliferative response to *C. albicans* cell-wall fractions (50 µg/ml). The response was assayed after the following treatments: A, medium only for 2 h, wash, culture for 6 days (control); B, M fraction 2 h, wash, culture for 6 days in medium; C, M fraction 2 h, wash, culture for 6 days in M fraction; D, GMP fraction 2 h, wash, medium culture for 6 days in medium; E, GMP fraction 2 h, wash, culture for 6 days in GMP fraction. Proliferation was measured on day 6 by the standard ³H-thymidine test. Bars represent ±SD.

Table III. Inhibitory effects of M and M-alk on CA-induced PBMC proliferation*

Material	Dose (µg/ml)	³ H-thymidine-incorporated (dpm × 10 ⁻³ ± SD)	
		without CA	with CA
none	—	0.7 ± 0.04	51.3 ± 0.9
M	100	7.8 ± 0.6	23.4 ± 5.7† (54)
M	50	11.2 ± 0.7	32.1 ± 6.8 (37)
M	25	11.7 ± 0.6	33.1 ± 4.4 (35)
M-alk	100	0.8 ± 0.06	24.0 ± 1.9‡ (53)
M-alk	50	0.9 ± 0.1	28.3 ± 2.5 (45)
M-alk	25	1.2 ± 0.05	29.7 ± 3.6 (42)

* Proliferation was assessed in triplicate samples each containing 2×10^5 PBMC. Cells were incubated for 1 h with the indicated fraction and then with CA 5 µg/ml until day 6, when ³H-thymidine incorporation was measured. Values in parentheses represent the percentage of inhibition with respect to CA-induced proliferation.

† $p < 0.05$.

‡ $p < 0.01$, when compared with the control (CA: first row, 'with CA' column).

Table IV. Response to CA and GMP antigens of human umbilical cord blood cells*

Material	Dose (µg/ml)	³ H-thymidine incorporated (dpm × 10 ⁻³ ± SD) by cells from	
		donor 1	donor 2
none	...	1.6 ± 0.22	4.60 ± 0.8
PHA	1 in 100 dilution†	92.6 ± 6.1	16.0 ± 2.52
CA	50	1.62 ± 0.51	—
	5	2.59 ± 0.42	3.4 ± 0.45
	0.5	1.91 ± 0.12	—
GMP-1‡	100	2.82 ± 0.20	—
	50	2.66 ± 0.46	—
	5	1.60 ± 0.71	—
GMP-2‡	50	2.06 ± 0.34	3.6 ± 0.05
	5	2.01 ± 0.16	4.8 ± 1.00
	0.5	1.80 ± 0.17	2.7 ± 0.50

* Proliferation was assessed by measuring ³H-thymidine incorporation on day 6 in triplicate samples containing 2×10^5 cord blood cells in a final volume of 0.2 ml/well.

† A 1 in 100 dilution of a commercial preparation.

‡ Two independent preparations of the same antigen.

mannan was shown to bind anti-*Candida* antibodies in the ELISA tests described. We cannot exclude the possibility that a small amount of protein or some undefined component in our preparation could have contributed to our M fraction acting as a blastogenic antigen.

We have shown that all mannan preparations inhibited *C. albicans* cell-induced lymphoproliferation. Fisher *et al.* (1982), working with *Candida* metabolic antigen as a stimulator and a mannan preparation similar to our M fraction as inhibitor, ascribed the inhibition of blastogenesis to a mannan-mediated interference with antigen presentation by adherent cells. This mechanism could even operate more efficiently when the lymphoproliferative stimulus is given by particulate *Candida*. However, considering the antigenic nature of the lymphoproliferation induced by GMP and M and the fact that *C. albicans* has mannan polysaccharide chains on its surface (Cassone *et al.*, 1978; Odds, 1979; Suzuki and Fukazawa, 1982), the capacity of mannan fractions to inhibit CA-induced lymphocyte blastogenesis could be due to competition for binding to responsive target cells, as also suggested by Nelson *et al.* (1984), for the inhibition by *Saccharomyces* mannan of *Candida*-antigen stimulated blastogenesis. Other explanations are possible including generation of suppressor cells (Piccolella *et al.*, 1981). The mechanism of inhibition and its specificity await further studies. Nevertheless, the fact that mannan preparations, even of different composition and structure, may

inhibit the proliferative response to *Candida* is a potentially relevant factor in the pathogenesis of candidosis, when consideration is given to the significant amount of mannan antigens secreted by the invading micro-organism in patients affected by systemic candidosis (De Repentigny and Reiss, 1984).

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