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Increased Sensitivity of Mannanemia Detection Tests by Joint Detection of α- and β-Linked Oligomannosides during Experimental and Human Systemic Candidiasis

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An enzyme immunoassay (EIA)—the commercially available Platelia *Candida* antigen test—developed for the diagnosis of systemic candidiasis is based on the detection of α-linked oligomannose residues (α-Man) released from *Candida* cells into the serum. This test has good specificity but has to be repeated frequently because of the rapid clearance of detectable mannanemia. We have developed a second EIA based on detection of beta-linked oligomannoses (β-Man), since β-Man are linked to different *Candida* molecules and interact differently with the host immune system and endogenous lectins and should therefore present different kinetics of serum clearance. In a guinea pig model of *Candida albicans* systemic infection, the relative amounts of detectable α- and β-Man differed considerably according to the virulence of the strain, the infecting dose, and the time after challenge that serum samples were drawn. Detection of α-Man was more sensitive per serum sample than that of β-Man, and the sensitivity for the combination reached 90%. The same tests were applied to 90 sera from 26 patients selected retrospectively for having been infected with the most-pathogenic *Candida* species: *C. albicans* (19), *C. tropicalis* (4), and *C. glabrata* (3). A total of 22 patients had positive antigenemia, 4 had α-mannanemia, 4 had β-mannanemia, and 14 showed the presence of both. For the patients showing the presence of both forms of mannanemia, the use of both tests enhanced the duration of the detection of mannanemia. Mannanemia was correlated with early clinical symptoms and isolation of *Candida* in culture, which occurred in 55% of the patients at an average of 4.7 days after the first positive mannanemia test result. A combination of the two tests had a cumulated specificity of 95%, and positive and negative predictive values were 79 and 97%, respectively. These findings provide evidence for different kinetics of β- and α-Man circulation during experimental and human candidiasis and suggest the joint detection of both types of epitopes as a rational approach contributing to increases in the sensitivity and earliness of diagnosis.

Systemic infections caused by *Candida* species are an increasing problem in clinical practice. Depending on the medical specialty, *Candida* species are the fourth or fifth most commonly recovered pathogens in blood cultures (27), with attributable mortality rates for candidemia of 40 to 60% (28, 41). The increasing incidence of systemic candidiasis and high mortality rates has been accompanied by escalating costs of prophylactic, preemptive, and empirical antifungal therapy (6, 7). These medical and economic problems are related to difficulties in establishing an early and specific diagnosis of infection (30, 31). The detection of several *Candida*-derived molecules in the serum samples of patients has been reported to be of diagnostic value. These molecules include metabolites (29, 43), proteins (25), nucleic acids (8, 22), and polysaccharides (either glucans or mannans) (25, 26, 33). Mannan is a large, complex, and highly immunogenic assembly of mannopyranose units linked through α-1,6, α-1,3, α-1,2, or β-1,2 bonds. It encompasses a large repertoire of antigenic oligomannose epitopes whose specificity is determined by the nature of the linkage between mannose residues and the length of the oligomannose chain (35). These epitopes are associated with a large variety of different yeast carrier molecules and may be species specific or shared by several species (3, 36–38). A clear-cut discrimination between α- and β-linked mannose residues is made by mammalian immune systems and endogenous lectins (11). The antibodies generated are highly specific for each category (15, 16, 24).

A large number of anti-mannan antibodies have been used for the development of antigen detection tests. For all of them, sensitivity is impaired by the rapid clearance by host catabolism of epitopes from the bloodstream (14, 21, 33). We have developed an enzyme immunoassay (EIA) for the detection of mannan and shown that its combination with detection of anti-mannan antibodies was useful for the early diagnosis of systemic candidiasis (32, 33, 44); these tests have been recently marketed as the Platelia *Candida* Ag and Platelia *Candida* Ab tests, respectively. The Platelia *Candida* Ag test detects an α-linked oligomannose residue (α-Man) epitope whose circulation was transient. As beta-linked oligomannose (β-Man)

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TABLE 1. Established characteristics of MAbs[a]

MAb	Isotype (origin)	Immunogen	Recognized *Candida* molecules	Minimal epitope(s)	Recognized *Candida* species
5B2	Monoclonal IgM	*C. albicans* strain VW32 infection with live cells	Mannan, phospholipomannan, mannoproteins	β-1,2 Mannobiose (homopolymers and heteropolymers)	*C. albicans* (serotypes A and B) *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*
EB-CA1	Monoclonal IgM rat-mouse Lou/C	*C. albicans* formaldehyde-fixed yeast cells	Mannan, mannoproteins	Mannopentaose with α-1,2 mannotetraose sequence at the nonreducing end	*C. albicans* (serotypes A and B) *C. glabrata*, *C. tropicalis*, *C. parapsilosis*

[a] Established MAb characteristics were described previously (1, 18, 19, 35, 36).

epitopes may be expressed on *Candida* molecules that differ from those expressing α-Man, it could be anticipated that these different families of epitopes and their respective carrier molecules have (i) different kinetics of release from cells of *Candida* spp. in tissues and (ii) different kinetics of catabolism by the host. The aim of this study was to examine whether the simultaneous detection of both α- and β-linked oligomannosaccharides could enhance the duration of detection of antigenemia. For this purpose, we used two monoclonal antibodies (MAbs) with different specificities. The first MAb, EB-CA1, recognizes α-1,2-linked oligomannose sequences with more than four residues (17, 19, 25) which are present in the acid-stable fraction of mannan as well as on a wide range of *Candida albicans* mannoproteins. They are also present in mannoproteins from *C. glabrata* and *C. tropicalis* and, at a lower level, from *C. parapsilosis* and *C. krusei* (19). This MAb was used in the commercial Pastorex *Candida* test and, more recently, in the Platelia test. MAb 5B2 reacts with β-1,2-linked oligomannosides which are present in both the acid-stable and acid-labile fractions of *C. albicans* mannan (18, 37). These epitopes are also present on mannoproteins of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* (2) and are highly expressed on the phospholipomannan, a glycolipid synthesized by *C. albicans*, *C. dubliniensis*, and *C. tropicalis* (18, 37).

Circulation of α- and/or β-1,2-linked oligomannosaccharides was first investigated in serum samples from guinea pigs experimentally infected with *C. albicans*. The diagnostic value of the EIA involving MAb 5B2 and MAb EB-CA1 (Platelia *Candida* Ag test) was then assessed retrospectively in 26 patients with systemic candidiasis caused by *C. albicans*, *C. glabrata*, and *C. tropicalis*, the most pathogenic species encountered in clinical practice.

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MATERIALS AND METHODS

MAbs. Information about the MAbs is summarized in Table 1. MAb EB-CA1 is an immunoglobulin M (IgM) directed against α-linked pentaose (19) which was produced after injection of Lou/C rats with heat-killed *C. albicans* yeast cells (13). This MAb was kindly provided by Bio-Rad (Marnes la Coquette, France). MAb 5B2 is an IgM generated in our laboratory following a heterologous fusion between mouse myeloma cells and lymphocytes from a rat infected with *C. albicans* strain VW32 (18). This MAb reacts with *C. albicans* antigenic factors 5 and 6 (18).

Experimental infection. Two series of 12 male Pirbright guinea pigs (weighing approximately 500 g) were infected by intravenous inoculation with one of two strains of *C. albicans* serotype A, VW32 (1) or B2630 (23) (isolate held at the Janssen Research Foundation). For each strain, grading doses of 20,000, 30,000, and 40,000 CFU per gram of body weight were used according to a method described previously (12). Sera collected on days 1, 2, 3, and 7 and plasma collected on days 14, 21, and 28 were stored at −40°C.

Patients. A total of 90 serum samples were selected retrospectively between January 1996 and December 1998 from 26 patients (9 females and 17 males; mean age, 56.6 years) with proven candidiasis due to *C. albicans* (19 patients), *C. glabrata* (4 patients), or *C. tropicalis* (3 patients). The average number of serum samples per patient was 3.46 ± 1.5 (Table 2). The following criteria were applied as retrospective selection rules: (i) fever nonresponsive to antibacterial therapy but responsive to antifungal therapy; (ii) positive culture for a *Candida* species from a normally sterile site (blood, bile, pericardial fluid, liver biopsy specimen, drain fluid, or wound); (iii) availability of serum samples within a range of 1 week before to 2 weeks after cultures tested positive; and (iv) analysis of the charts of patients with special attention to risk factors.

In addition to fever, other clinical signs included endophthalmitis (patients B4 and D11) and cutaneous lesions (patients B4, D10, and D11). Curative antifungal management was instituted for all patients except for patients C1 and B1, who died the day after isolation of a *Candida* sp. from blood cultures. For all patients except D8, broad-spectrum antibiotics were first administered an average of 2 weeks before isolation of a *Candida* sp. In 23 patients, central venous catheters or peripheral venous access ports were in situ at the time of positive blood culture results. Neutropenia (<500 leukocytes/mm^3) in seven patients (B3, B4, C4, D2, D4, and D12) and oral or intravenous immunosuppressive therapy (corticosteroids in 14 patients) were also documented. Five patients had diabetes mellitus.

Control sera. Five groups of control sera were included in this study. Group 1 consisted of the serum samples of 15 patients at high risk for systemic candidosis who were hospitalized in intensive care units (ICU) or surgery or respiratory wards and for whom *Candida* colonization was documented at two or more body sites but who presented no evidence of *Candida* infection. Group 2 comprised the serum samples of 10 patients with deep fungal infection not caused by *Candida* (7 patients with invasive pulmonary aspergillosis and 3 patients with cryptococcal meningitis). Group 3 consisted of 10 sera from nine patients with high levels of *Cytomegalovirus* antigenemia. Group 4 consisted of sera drawn from 70 healthy blood donors. Sera from 14 patients with high levels of rheumatoid factor (known to generate interference with serological tests) were included as group 5.

Serological tests. Detection of circulating α- and β-linked oligomannosides was performed.

Detection of α-1-2-linked oligomannosides. The method employed was based on a commercially available Platelia *Candida* Ag test (Bio-Rad) (33). This one-step, sandwich, microplate EIA uses the MAb EB-CA1 (3.4 mg/ml) as a captor and detector antibody to allow the detection of mannan in serum samples. Each patient's serum sample (300 μl) was treated with 100 μl of dissociating solution, boiled for 3 min, and centrifuged for 10 min at 10,000 × *g* to release free antigens. Supernatant (50 μl) was added to EB-CA1-precoated microtiter plate wells with 50 μl of horseradish peroxidase (HRP)-conjugated MAb EB-CA1. After incubation for 90 min at 37°C, the plates were washed thoroughly and the reaction was detected by incubation with 200 μl of tetramethylbenzidine solution for 30 min according to the manufacturer's instructions. Each experiment included a calibration curve obtained with a pool of normal human sera spiked with different concentrations of mannan in the range of 0.1 to 2 ng/ml.

Detection of β-1-2-linked oligomannosides. A similar method was developed for the detection of circulating β-1,2-linked oligomannosides. Polystyrene microtiter plates were coated with 100 μl of MAb 5B2 (1 μg/well). The subsequent steps were performed as described above except for the detection step, which was performed with HRP-conjugated MAb 5B2 for the homologous system (MAb 5B2 as captor and detector) or HRP-conjugated EB-CA1 for the heterologous system.

TABLE 2. Underlying diseases and culture data for infected patients

Patient	Sex	Age	Hospital ward	Underlying condition	Site of yeast isolation	Isolated species	No. of sera	Antifungal therapy
A1	F	42	Surgery	Liver transplantation	Blood	*C. albicans*	4	FCZ[g]
A2	M	68	Gastroenterology	Pancreatic cancer	Blood	*C. albicans*	4	FCZ
A3	M	45	ICU	Cardiopulmonary arrest	PL[d] (drain)	*C. albicans*	3	FCZ
A4	M	79	ICU	Respiratory decompensation	Blood	*C. glabrata*	3	FCZ + AmB[h]
B1	M	66	ICU	Respiratory decompensation	Blood	*C. albicans*	5	None[i]
B2	M	37	Transplantation	Liver transplantation	BAL[e]	*C. albicans*	4	FCZ
B3	M	46	Hematology	MNHL[a]-ABMT[b]	Blood	*C. tropicalis*	3	AmB
B4	M	60	Hematology	AML[c]	Blood	*C. tropicalis*	7	AmB
C1	F	65	ICU	Acute pulmonary edema	Blood	*C. albicans*	2	None
C2	M	64	ICU	Acute pancreatitis	Blood	*C. albicans*	4	FCZ
C3	F	58	ICU	MNHL	Blood	*C. glabrata*	4	FCZ + AmB
C4	M	75	ICU	Febrile aplasia	BAL	*C. tropicalis*	2	AmB
D1	M	73	ICU	Pneumopathy	PBB[f]	*C. albicans*	1	FCZ
D2	M	17	ICU	AML-ABMT	Blood	*C. albicans*	2	FCZ + AmB
D3	F	62	ICU	Bronchopneumonia	BAL	*C. albicans*	4	AmB
D4	M	31	ICU	MNHL	Blood	*C. albicans*	4	FCZ
D5	M	76	ICU	Bilateral pneumopathy	BAL	*C. albicans*	3	AmB
D6	M	47	ICU	Cerebrovascular accident	BAL	*C. albicans*	5	FCZ
D7	F	70	ICU	Hyperthermia	Blood	*C. albicans*	5	AmB
D8	M	71	Surgery	Pancreatectomy	Blood	*C. albicans*	1	FCZ
D9	F	46	Surgery	Acute pancreatitis	Blood	*C. albicans*	2	FCZ
D10	F	47	Surgery	Appendicectomy	Blood	*C. albicans*	2	FCZ
D11	M	48	Surgery	Ulcerative colitis	Blood	*C. albicans*	4	FCZ
D12	M	51	Hematology	AML	Blood	*C. albicans*	4	FCZ
D13	F	64	ICU	Acute respiratory distress	Blood	*C. glabrata*	4	FCZ + AmB
D14	F	65	Surgery	Acute pancreatitis	Blood	*C. tropicalis*	4	FCZ

[a] MNHL, malignant non-Hodgkin's disease lymphoma.
[b] ABMT, allogeneic bone marrow transplantation.
[c] AML, acute myelogenous leukemia.
[d] PL, pleural liquid.
[e] BAL, bronchoalveolar lavage fluid.
[f] PBB, peribronchial biopsy.
[g] FCZ, fluconazole. Patients received 400 mg per day as a loading dose and 200 mg per day as a maintenance dose.
[h] AmB, amphotericin B. Depending on renal function, AmB was given at 0.5 to 1 mg/kg of body weight per day.
[i] None, no therapy was administered.

For MAb 5B2 conjugation, HRP (Boehringer) in HEPES buffer was activated by oxidation with sodium periodate (39). The activated peroxidase was then purified by column chromatography on PD10 Sephadex (Pharmacia Biotech, Uppsala, Sweden), diluted in phosphate-buffered saline, and subsequently incubated for 3 h with the MAb (enzyme/antibody ratio, 25/1 mol). The conjugated MAb 5B2 was dialyzed using phosphate-buffered saline and stored at 4°C in a solution of 0.01% sodium merthiolate. The optimal concentration of HRP-conjugated MAb 5B2 (determined in an EIA using a graded concentration of mannan) was 8 μg/ml.

RESULTS

Detection of α- and β-linked oligomannosides in sera from experimentally infected guinea pigs. Figure 1 shows individual antigenemia values for each serum sample drawn from 24 guinea pigs intravenously injected with different doses of two *C. albicans* strains. Mortality was strain and inoculum dependent; at the end of the experiment (day 28), the overall mortality rates were 25% (three animals) and 58% (seven animals) for strains B2630 and VW32, respectively (Spearman correlation; $P < 0.030$). Both α- and β-oligomannoside epitopes could be detected in each of the infected animals. However, Figure 1 shows that among the 125 sera available, there was considerable variability in the quantity of antigen (0.1 to 9 ng/ml) detected for each epitope; the quantity depended on the strain, the challenge dose of *C. albicans*, and the day the sera were collected. For guinea pigs infected with strain B2630, 92% of sera presented results positive for α mannanemia and 78% presented results positive for β mannanemia. The values for both mannanemias were maximal during the first week after infection, became negative or weakly positive, and then increased again by day 28. For animals infected with strain VW32, only 56 sera were available due to mortality and disseminated intravascular coagulation. Variations in antigen levels were observed in successive serum samples drawn from the same animal. Although 82% of the serum samples were positive according to the results of at least one test, the sensitivity was 79 and 46% for α- and β-mannanemia. The amount of α-linked residues was higher than that of β-linked residues in 48% of the serum samples. For 6% of the serum samples, the quantity of β-linked residues was larger than that of α-linked residues.

Detection of *Candida*-derived oligomannosides in control and patients' sera. The results for mannanemia detection tests are shown in Table 3. Patients were classified into four groups which correlated with their serologic patterns. In the first group (patients A1 to A4; $n = 4$ [15.3%]), no antigenemia was detectable at any time despite the availability of several sera

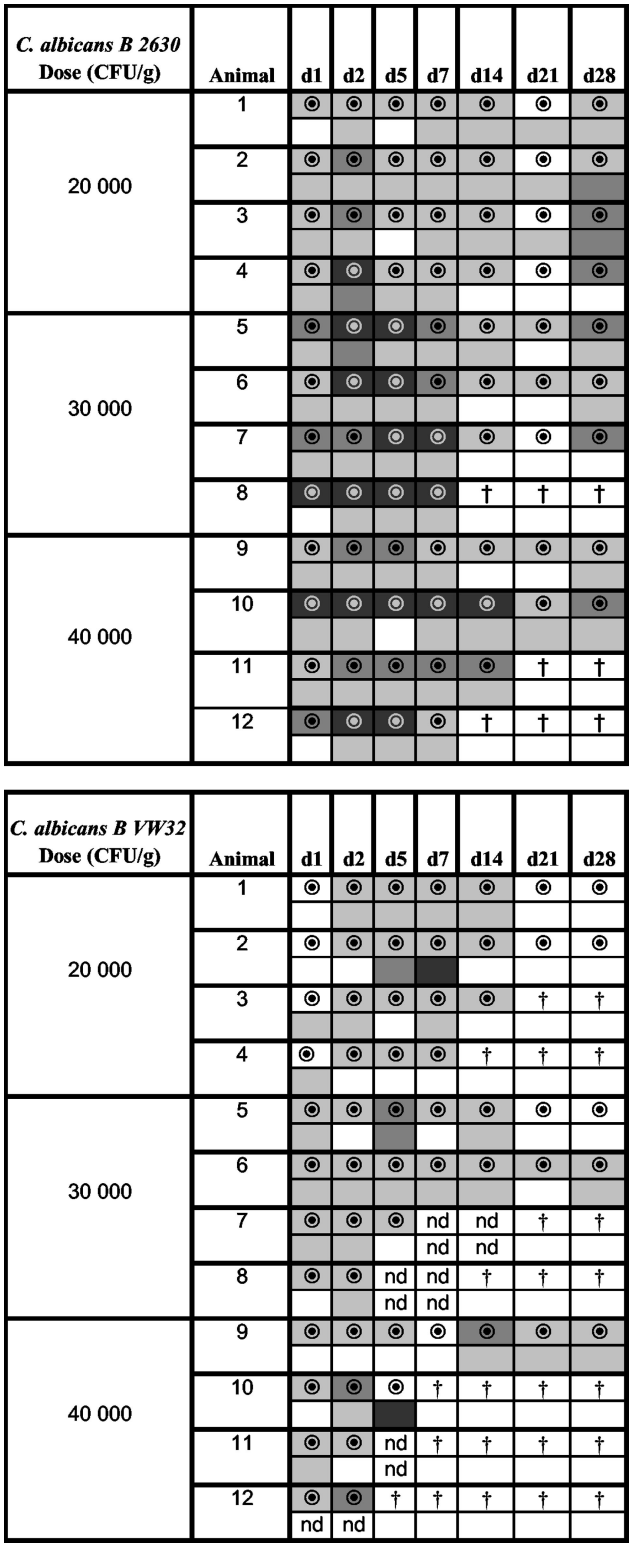

FIG. 1. Kinetics of circulating α- and β-linked mannoglycoconjugates as detected by MAb EB-CA1 (Platelia Ag) or MAb 5B2 (EIA 5B2) (boxes with or without ● symbols, respectively) in sequentially drawn serum samples from guinea pigs experimentally infected by two strains of *C. albicans*. For each test, the levels of α- and β-mannanemia were graded from minimal (mannanemia levels of <0.1 ng/ml [white boxes]) to peak (mannanemia levels of ≥1 ng/ml [black boxes]) on the basis of the detected mannan concentrations; intermediate values are represented by light-grey box shading (mannanemia levels between ≤0.1 ng/ml and <0.5 ng/ml) or dark-grey box shading (mannanemia levels between ≤0.5 ng/ml and <1 ng/ml). nd, tests not done; †, deceased animals.

TABLE 3. Results of antigen and antibody testing in patients with candidiasis

Patient	Result of:					
	Platelia *Candida* Ag test (ng/ml)		EIA β-Man 5B2/5B2 captor-detector test (ng/ml)		EIA β-Man EB-CA1/5B2 captor-detector test (ng/ml)	
	Peak	Mean	Peak	Mean	Peak	Mean
A1	0	0	0.4	0.1	0	0
A2	0	0	0	0	0	0
A3	0	0	0	0	0.4	0.2
A4	0	0	0.4	0.1	0.4	0.1
B1	0.6	0.2	0	0	0	0
B2	0.5	0.2	0	0	0	0
B3	0.6	0.2	0.4	0.1	0	0
B4	2	0.6	0.4	0.1	0.4	0.05
C1	0	0	2	1.5	0.7	0.4
C2	0	0	2	0.5	2	0.6
C3	0	0	0.7	0.2	0	0.2
C4	0.4	0.2	0.5	0.3	0.6	0.3
D1	2	2	2	2	2	2
D2	1.2	1.1	0.8	0.7	0.5	0.5
D3	0.8	0.2	1.1	0.5	1.1	0.3
D4	0.7	0.5	2	0.5	0	0
D5	2	1.3	2	1.3	2	1.2
D6	0.7	0.5	1.6	0.7	1.2	0.7
D7	0.7	0.3	2	1.2	0.6	0.2
D8	2	2	2	2	2	2
D9	2	1	1.2	0.6	2	1
D10	1.5	0.8	1	0.7	0.6	0.5
D11	1	0.25	1	0.25	1	0.25
D12	2	1	0.4	0.1	1	0.4
D13	0.6	0.2	0.5	0.1	0	0
D14	2	0.6	2	0.7	2	0.6

from each patient. In the second group (patients B1 to B4; n = 4 [15.3%]), β-mannanemia results were negative throughout but a significant α-mannanemia result was detected. In the third group (patients C1 to C4; n = 4 [15.3%]), β-mannanemia was detected in at least one sample but without evidence of α-mannanemia. For 54% of the patients (group D), both antigenemia detection test results were positive for a given patient (although usually not in the same serum sample). A representative example of the differences observed in the time course of α- and β-mannanemia detection in such patients is shown in Fig. 2. For this patient (D7) (Table 2 and Table 3), Platelia Ag test (α mannanemia) and β-mannanemia test results were positive 5 days before the positive-testing blood culture was drawn (and 7 days before *C. albicans* grew in culture). α-Mannanemia results then became consistently negative, whereas a peak of β-mannanemia, which was coherent with the persistence of an infectious syndrome, was observed 2 weeks after the sample giving a positive blood culture result was drawn.

Figure 2 also illustrates how the use of heterologous detection systems gave insights into the complex nature of circulat-

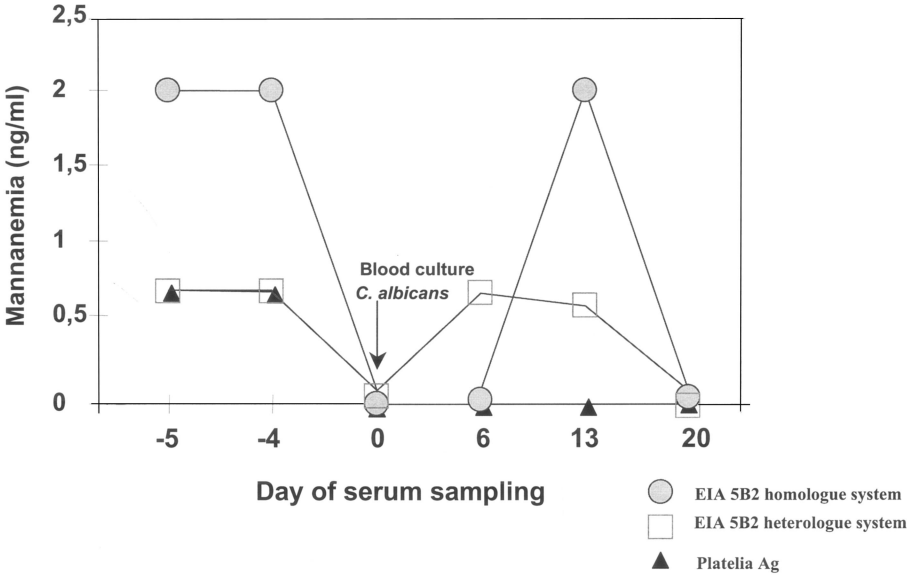

FIG. 2. Example of kinetic evolution of mannanemia as detected by different tests. Patient D6 had systemic candidiasis; the date of mycological isolation of *C. albicans* from blood culture is indicated by the arrow.

ing *Candida* antigens. Although sera drawn on both day 6 and day 13 gave negative results with MAb EB-CA1 in the homologous system (Platelia Ag) for detection of α-Man (when the same MAb was used as a captor and MAb 5B2 [anti-β-Man] was used as a detector), antigenemia was detected. Similar results were obtained with sera from patients C1 and C4. This means that in antigens released from *C. albicans*, both α- and β-Man epitopes were associated. These antigens were detectable in host serum, although the level of α-Man repetitive epitopes alone was not high enough to allow their detection. Interestingly, mannanemia could be detected before culture positivity. α-Mannanemia was detected in 62% of patients at an average of 5.6 days before cultures gave positive results, and β-mannanemia was detected in 48% patients at an average of 3.9 days before cultures gave positive results.

Controls consisted of a panel of representative hospitalized patients, including (i) patients at high risk for systemic candidosis who were hospitalized in clinical wards and for whom *Candida* colonization was documented but who presented no evidence of *Candida* infection, (ii) patients with deep fungal infection not caused by *Candida* spp. (including patients with invasive pulmonary aspergillosis, cryptococcal meningitis, and cytomegalovirus infection), (iii) patients with high levels of rheumatoid factor (known to generate interference with serological tests), and (iv) healthy blood donors. The results obtained with these control sera are summarized in Table 4. Depending on the tests, positive antigenemia (at low levels) results were observed for 2.5 to 5.6% of control sera. Most of the patients with low-level results had several colonized body sites, a context that cannot exclude the presence of a subclinical *Candida* infection. This choice of control population probably accounts for the relatively low cumulative predictive positive value of the tests (79%) (Table 5).

Altogether, the results obtained showed that the combination of the Platelia Ag test and 5B2 EIA increased the sensitivity of mannan detection from 69 to 85%; their negative predictive value was 95%.

DISCUSSION

In this report we demonstrate that α- and β-oligomannosides synthesized by *Candida* species have different kinetics of

TABLE 4. Results of antibody and antigen testing on sera from control populations

Category	No. of patients	No. of sera	No. of sera that tested positive[a]		
			Platelia *Candida* Ag test	EIA 5B2 homologous system	EIA 5B2 heterologous system
Intensive care patients without invasive candidiasis	15	44	1	5	1
Aspergillosis	7	7	0	0	0
Cryptococcosis	3	3	0	0	0
Cytomegalovirus infection	9	10	2	2	2
Healthy blood donors	70	70	0	0	0
Patients with rheumatoid factor activity	14	14	0	0	0
Total	118	148	3 (2)	7 (4.7)	3 (2)

[a] Values in parentheses are percentages.

TABLE 5. Sensitivity, specificity and predictive values for the detection of α- and β-mannanemia detection tests[a]

Parameter	Value (%) for test		
	Platelia *Candida* Ag	EIA 5B2 homologous system	Combination of both tests
Sensitivity	69	69	85
Specificity	98	95	95
Positive predictive value	86	75	79
Negative predictive value	94	94	97

[a] Results (per patient) were calculated following an analysis of 90 serum samples from 26 patients with systemic candidiasis and 148 sera from 118 controls.

circulation during experimental and human candidosis. This was evidenced by the use of sensitive immunoassays involving MAbs, each previously characterized for its specificity for an epitope type (18, 19) and for the nature of the structural *Candida* molecules carrying the respective epitopes (1, 37). Both epitopes were distributed over a large variety of molecules expressed by the major pathogenic *Candida* species (13, 19). Such a high level of expression is a necessary condition for sensitive detection.

Since the initial observation by Weiner and Coasts-Stephen (40), a large number of studies have been designed to examine detection of mannan in serum for diagnostic purposes (9, 24, 42). However, with few exceptions, the nature of the epitope detected in these studies was unknown. Thanks to progress made in epitope determination, it can be now concluded retrospectively that both α- and β-mannosaccharides released by *Candida* spp. can be detected (14, 19, 35, 37). Whatever the specificity of the antibody used, a rapid clearance of detectable antigen impaired the sensitivity of the tests (5, 21). Considering that the α- and β-Man epitopes detected here may be expressed on the same carrier molecule but also on different molecules, we hypothesized that their release in the infected host, their uptake by host endogenous lectins and antibodies, and their catabolism occur at different rates. This was observed in both experimentally infected animals but also in sera from patients infected by *Candida* species. Use of a combined system for detection of multiple epitope types thus led to increasing antigen detection sensitivity that compensated for the transitory nature of individual residues in circulation.

No conclusion can yet be drawn concerning the pathophysiological significance of each type of mannoside residue in circulation. In animals there was an obvious dose dependence relationship between fungal load and antigenemia for the less pathogenic strain. In individual animals the kinetics were similar: high antigenemia levels following intravenous injection and bloodstream dissemination, a relative decrease observed when fungal growth begin to develop in tissues, and—for animals that survived further—another late peak of antigenemia seen at the stage at which their organs were deeply invaded and their condition deteriorated. Interestingly, infection with the more virulent *C. albicans* strain (which was presumably eliminated in lower quantities by host defenses) led to lower levels of antigenemia. This conclusion is not definitive, since rapid death and disseminated intravascular coagulation in this group of animals limited the availability of sera from later stages of infection. High variability of results between animals infected by the same strain at the same dose probably reflects individual susceptibility differences to *C. albicans* challenge.

In patients, our results also showed that the circulation characteristics of α-Man and β-Man epitopes were different for sera drawn successively from the same patient. Sera from four patients (C1 to C4) displayed only β-Man antigenemia, whereas only α-Man antigenemia could be detected in sera from patients B1 to B4 (Table 3). The Platelia *Candida* Ag assay was positive for 18 (69%) of the 26 patients with systemic candidiasis. The two versions of the test involving MAb 5B2 (homologous and heterologous systems) had sensitivity levels of 69 and 58%, respectively. In several cases, patients displayed positive results with only one test. The association of all three tests would have allowed a diagnosis of candidiasis in 85% of the patients. The results obtained by the heterologous system (MAb EB-CA1 as captor and MAb 5B2 as detector) revealed circulating macromolecules which expressed both α- and β-Man epitopes, although the level of α-Man repetitive epitopes alone was not high enough to allow their detection. The reciprocal heterologous test was also tried (MAb 5B2 as captor and MAb EB-CA1 as detector), and no significant difference in sensitivity was observed.

All antigen detection tests displayed good specificity, even though specificity was well challenged by a control population chosen to include many individuals likely to induce false-positive reactions. This population consisted of patients suffering from other deep-seated mycoses (invasive aspergillosis, cryptococcosis) or afebrile hospitalized patients known to be colonized with *Candida* species but showing no mycological evidence of *Candida* infection. The best performance of the tests was their negative predictive value (97%), which is of interest to those in clinical practice facing situations in which the majority of patients present multiple risk factors for developing a *Candida* infection. As stated recently in a review by Stevens (34), serological tests of high negative predictive value could be of help to identify those who do not require prescription of antifungals.

An alternative strategy for diagnosis of systemic candidiasis is PCR, which has recently been found to give promising results. This method is based on the amplification of more or less specific portions of DNA sequences from *Candida* species whose presence in blood or serum was shown to be specific for infection (4, 8, 22, 29). In a preliminary study, Sendid et al. showed that serology and PCR contribute equally to the diagnosis of *Candida* infections proved by autopsy (E. Norberg, L. Klingspor, B. Sendid, L. Dumortier, N. Francois, J. Tollemar, and D. Poulain, Abstr. 6th Trends Invasive Fungal Infect., Prague, abstr. P36, 18-20 October, 2001). In the same way as for antigens, the physiology and kinetics of the release and degradation of nucleic acids from infecting fungi are still unknown. Although diagnostic sequences may encode essential yeast genes and can be easily amplified, these molecules are pathophysiologically inert. Antigens, by contrast, can (by definition) induce an antibody response, and a clear-cut balance between high anti-mannan antibody levels and mannanemia in sera from patients with systemic candidiasis led us to propose a strategy based on a joint detection of both antigen and anti-mannan antibodies. Through the same logic as that developed here for antigen detection, however, it is now well established that anti-mannan antibodies are directed against a mix-

ture of epitopes including α- and β-Man homopolymers or heteropolymers. Moreover, it has been experimentally shown that anti-β-Man antibodies were protective whereas anti-α-Man antibodies were not (15, 16, 24). Interestingly, Joualt et al. have shown that in patients with *Candida* infection, but not in patients colonized with a *Candida* sp., there was a down regulation of anti-β-Man "protective" antibodies which was not observed for the "nonprotective" anti-α-Man antibodies (20). At the moment, the Platelia Ab test detects antibodies against the whole mannan oligomannose repertoire containing both epitope types (33). Studies are in progress for developing multiparametric tests involving *Candida* oligomannose synthetic analogues to detect separately antibodies specific for different types of residues (10). Together with joint detection of the same residues, this would provide a coherent model with the goal of gaining a better understanding of the pathophysiology of this disease together with a higher sensitivity level for the diagnostic or even prognostic significance of the tests.

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