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Characterization with Crossed Immunoelectrophoresis of Some Antigens Differentiating a Virulent *Candida albicans* from Its Derived, Avirulent Strain (42552)

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Abstract. Antigenic differences between a wild-type virulent *Candida albicans* 4918 (*wt*) and its spontaneous avirulent mutant (*m-10*) were found with crossed immunoelectrophoresis. Yeast cell extracts as well as soluble protein and mannoprotein fractions obtained by affinity chromatography on concanavalin A (Con A) were analyzed. Sera from patients with candidiasis and antisera from rabbits infected with live *wt* cells and boosted with *wt* extracts or rabbits immunized with purified *wt* cell wall preparation were used as counter reactants. Qualitative differences in serum precipitins formed by patients with suspected or culture-proven candidiasis to polysaccharide antigens of *wt* and *m-10* origin were observed. In comparison, except for a spike-formed precipitate detected only with the *wt* extract, the serum from infected rabbits precipitated the *wt* and *m-10* cell wall polysaccharide antigens about equally. The same type of precipitate was also found with the Con A *wt* mannoprotein fractions but was again lacking with the *m-10* mannoproteins. This precipitate, with extremely slow electromobility in the first dimension, may be related to some special immunodeterminant of the *wt* mannan molecule. No substantial differences in the precipitation patterns of the Con A *wt* and *m-10* proteins were found when analyzed with patients' sera or rabbit anti-cell sera. However, using these protein fractions with anti-cell wall sera revealed a larger number of precipitates for the *wt* as opposed to the *m-10* strain. The observed antigenic differences between the virulent- and the avirulent-derived strains seem to be mainly associated with cell wall determinants (components) and might be related to the greater adherence and infectivity of the wild strain.

Adherence of *Candida albicans* to traumatized valvular endocardium is necessary for the establishment of endocarditis in a rabbit model (1, 2). Nonpathogenic *Candida* such as *C. krusei* (1, 3), as well as spontaneously derived, avirulent mutants of *C. albicans,* are unable to adhere *in vitro* to fibrin–platelet matrices similar in appearance to the matrix which forms on the valvular endocardium in response to trauma. Thus, nonadhering strains of *C. albicans* or other *Candida* species do not cause endocarditis as does the wild-type *C. albicans.*

The biochemical nature of the *Candida* surface component which recognizes damaged endocardium is unknown, although a mannoprotein has been implicated indirectly in the following way (4). Sheep red blood cells (SRBC), coated with *C. albicans* mannoprotein which was pretreated by acetolysis or mannosidase, did not attach *in vitro* to fibrin–platelet matrices. In addition, Pronase digestion of mannoprotein had no effect on the attachment of coated SRBC (4). With regard to adherence to mammalian cells, others have suggested that a mannoprotein (or mannan) promoted the attachment of *C. albicans* to vaginal (5) or buccal epithelial cells (6), although several investigators have shown that vaginal epithelial cells preincubated with chitin (or amino sugars) did not bind yeast cells of *C. albicans* (7). Thus, the nature of the *Candida* receptor, especially for epithelial cells, remains unknown.

We have isolated spontaneous mutants of *C. albicans,* unable to adhere *in vitro* or cause endocarditis in the rabbit model, and we were interested in determining whether alterations in cell-surface adherence properties are accompanied by antigenic changes. To our knowledge no comparative serological studies on such mutant wild-type pairs have been reported. In the present study, one of two mutants, isolated originally for its resistance to the antibiotic cerulenin and avirulence (*m-10*), was selected for comparative serological analyses with the parental virulent, wild-type *C. albicans* (*wt*). The approach taken in the present study was directed toward differentiation

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of antigenic components in the total cell extracts as well as candidal proteins and mannoproteins separated by affinity chromatography. Crossed immunoelectrophoresis using patient sera and hyperimmune rabbit sera made against the *wt* strain was employed.

Materials and Methods. *Candida albicans.* Strain 4918, a virulent isolate from a patient (8), was obtained from Dr. Thomas Mitchell, Duke University, Durham, North Carolina. Strain *m-10,* a spontaneous, cerulenin-resistant mutant of the *wt* 4918 was described earlier (9).

Antigens. Yeast cell extracts of the 4918 (*wt*) and 4918-10 (*m-10*) strains were prepared as follows: A 16-hr culture at 25°C in phytone–peptone broth containing 0.1% glucose was used as inoculum at 1×10^7 cells per milliliter for 250 ml of medium consisting of 1% yeast extract, 1% peptone, and 2% dextrose. The cultures were incubated on a gyratory shaker (150 rpm) for 48 hr at 25°C and consisted of greater than 95% yeast cells. Cells were harvested by centrifugation, washed extensively with deionized water containing 1 m*M* PMSF, and disrupted with glass beads (0.5 mm) with a hand vortex homogenizer. Cooling was effected by periodically immersing the cell suspension in an ice bath. Breakage was monitored with phase microscopy to attain 95%.

The cell homogenate was centrifuged at 10,000*g* for 20 min, at 25,000*g* for 20 min and finally at 100,000*g* for 1 hr. A clear supernatant was then concentrated to $\frac{1}{3}$ vol by ultrafiltration (Millipore CX-10), membrane filtered, and stored in aliquots at −20°C. They were designated *wt* and *m-10* extracts and were used in the serological analyses of the patients' and rabbit sera.

For affinity chromatography and immunization purposes, 100 ml of the final supernatants was brought to 70% saturation with $(NH_4)_2SO_4$ and further processed according to Ellsworth *et al.* (10) with a final lyophilization step.

Concanavalin A fractionation. The lyophilized extracts were subjected to affinity chromatography on concanavalin A (Con A) (Con A–Sepharose R 4B; Pharmacia Fine Chemicals, Uppsala, Sweden) performed essentially as described by Ellsworth *et al.* (10). A 200-mg sample of lyophilized material, dissolved in the primary buffer, was separated on a 0.7 by 18-cm column at 4°C at a flow rate of 8 ml/hr. Two fractions, the flow-through soluble protein and the bound mannoprotein complex, which was eluted with 0.01 *M* phosphate buffer containing 0.2 *M* α-methyl mannoside, were dialyzed against PBS or distilled H_2O, respectively, and lyophilized. They were designated *wt prot* and *m-10 prot* or *wt mann* and *m-10 mann.*

Total protein. Total protein was estimated according to Lowry *et al.* (11) with bovine serum albumin as a standard. The phenolsulfuric acid technique (12) was employed for determination of total carbohydrate using mannose as a standard.

Antisera. Patient's sera. Sera from four patients with culture-proven candidiasis (Group I) were kindly provided by Dr. Roy Hopfer, M.D., Anderson Tumor Institute, Houston, Texas. They had latex agglutination titers between 1:16 and 1:64. The Group II sera from 11 patients with suspected candidiasis, with antibody titers of 1:8–1:64 according to counter electrophoresis, were obtained from Dr. Margaret F. Price, Baylor College, Department of Internal Medicine, Houston, Texas.

Rabbit immune sera. The immunogens employed were from the *wt* strain. Antibodies to cellular antigens were developed by infecting intravenously New Zealand white rabbits with 2×10^5 live cells grown for 18 hr on BHI agar at 37°C. The rabbits were reinfected with the same dose of live cells after 2 weeks and bled 3 to 4 weeks later. These sera, with agglutinin titers of 1:80 to 1:160, were used for comparison with the patient sera. Agglutinins were performed according to the procedure of Reiss *et al.* (13).

The antibody levels in the infected rabbits were increased by booster injections of the lyophilized *wt* extract: 2 mg of protein/1 ml of 0.1 *M* NaCl was mixed at a 1:1 ratio with Freund's incomplete adjuvant. The antigen–adjuvant mixture (0.1 ml) was injected subcutaneously into each shoulder of the rabbits at 10-day intervals. The rabbits were bled after the seventh injection and the serum was designated *anti-cell* serum. *Anti-cell wall* sera were also produced in New Zealand white rabbits using cell wall from the *wt* strain obtained by the procedure of Reiss *et al.* (13). The immunization scheme was as follows: A dose of 0.5 mg in 0.2 ml PBS (lyophilized pu-

rified cell wall) was injected intravenously three times weekly for 4 weeks. Then the scheme was repeated four times at 3-month intervals. The anti-cell wall serum used in this study was produced by injecting a total of 30 mg purified cell wall preparation.

All rabbit and patient sera were initially examined using immunodiffusion and compared to preimmune rabbit and normal human sera.

Crossed immunoelectrophoresis. The Laurell technique (14) with an intermediate gel (15–17) was utilized. Electrophoresis was performed in 1% agarose (Bio-Rad) in Tris–barbital buffer, pH 8.6, ionic strength 0.02. The *wt* and *m-10* antigen samples, adjusted to 25 μg of protein, were usually run simultaneously on a single plate (7 × 10 cm). The first dimension separation was 7 V/cm, 12°C, for about 50 min, depending on the migration of a bromphenol blue albumin marker. After electrophoresis, an intermediate gel (1.5 cm wide) with or without a reactant and an upper gel containing 18 μl antiserum/cm^2 were applied. The second dimension run was overnight at 2 V/cm at 12°C. Nonprecipitated proteins were eluted by alternately pressing and swelling the gel with PBS. Subsequently, the plates were air-dried and stained for 10 min in 2% Coomassie brilliant blue R in methanol:glacial acetic acid:water (45:10:45). Excess stain was removed by destaining in the same solvent at 25:10:65 proportions.

Results. The protein and carbohydrate content of the antigen preparations from *wt* and *m-10* were *wt extract,* 56 and 8.6%; *m-10 extract,* 55 and 8.8%; *wt prot,* 42 and 0.3%; *m-10 prot,* 39 and 0.5%; *wt mann,* 16% and 52.5%; *m-10 mann,* 15 and 51.5%.

Immunodiffusion analysis with either cell extracts or the Con A mannoprotein fraction of *wt* and *m-10* strains showed an increased reactivity to cell wall determinants in precipitation patterns with the anti-cell wall serum versus anti-cell serum. With the Con A protein fractions, multiple line spectra were formed with the anti-cell serum in comparison to only a few lines with the anti-cell wall serum. Patient sera generally reacted strongly with the *wt* extracts but showed variation in reactivity with the *m-10* extracts. Preimmune rabbit sera and normal human sera formed no precipitin lines in immunodiffusion analyses with any of the antigen preparations.

With crossed immunoelectrophoresis (XIE), the antigen molecules were separated in the first dimension according to their electromobility. In the second dimension, on traveling into the antiserum–agarose, the separated antigens formed precipitate peaks which permitted their characterization and the identification of corresponding precipitins according to established principles (16). By utilizing intermediate gels containing different antisera as well as plain agarose (control), their precipitin content could be interrelated and compared to the anti-*wt* cell serum in the upper gels.

Whole cell extracts were initially used to study differences between the *wt* and the *m-10* strains. Figure 1a illustrates the precipitation patterns for the *wt* and *m-10* extracts obtained with an antiserum produced by infecting rabbits with live cells of the *wt* strain. The patterns for both extracts contain broad, heavily stained lines developed by antigen(s) with moderate but extended electromigration. The *wt* line coalesced with a spike of precipitate formed by molecules having very slow mobility which were not detected in the *m-10* extract. (See arrow, Fig. 1a.)

The presence of two populations of antigens with common determinants but unlike electromobilities in the *wt* extract was revealed in the intermediate gel by reaction with sera from patients with culture-proven candidiasis (Group I), as represented in Fig. 1b. The pattern shows two broad precipitates, labeled with arrows 1 and 2, extending from the spikes near the *wt* start basin formed by precipitins at different but high concentrations in the patient's serum. No spikes were seen in the otherwise similar *m-10* spectrum.

The patient's serum also contained precipitins to an antigen with fast electromobility (most anodic) at a higher concentration in the *wt* extract compared with the *m-10,* as indicated by the area beneath the peaks. The precipitate extensions into the intermediate gel from the major as well as some minor peaks in the spectra formed in the upper gel indicated additional reactive antibodies at low concentrations in the patient's serum.

To further characterize the broad, dense, heavily stained precipitates with moderate electromobility, mannoprotein fractions obtained by Con A affinity chromatography of

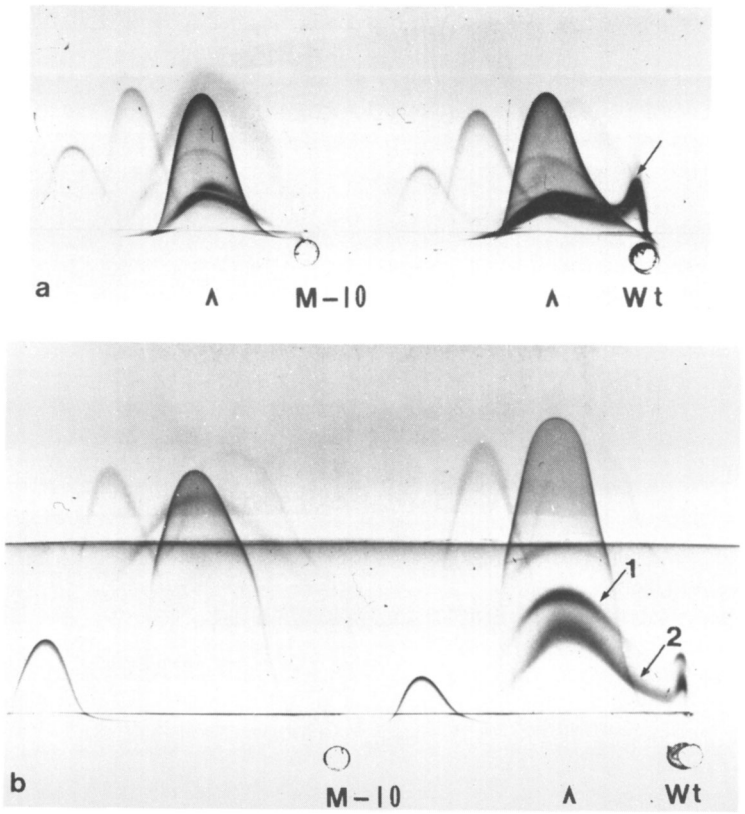

FIG. 1. Crossed immunoelectrophoresis analyses of *C. albicans* whole cell extracts *m-10* and *wt*. (a) Serum from infected rabbits in the second dimension gel. (b) The same reactants in upper gel as those in (a) with an intermediate gel containing serum from a Group I patient. Note the broad, intensely stained precipitates in the spectra for *m-10* and *wt* in (a), but revealed in (b) as two precipitates (arrows 1 and 2) coalescing with spikes above the start (O) only with *wt*. The anode is left for the first dimension and top for the second in all of the figures. Open arrows at the bottom of Figs. 1 and 2 indicate the peaks of precipitate with moderate electromobility.

the *wt* and *m-10* extracts were tested against a hyperimmune anti-cell serum, with a patient serum (Group II), Con A, or anti-cell wall serum in the intermediate gels, see Figs. 2a, 2b, and 2c. The pattern in Fig. 2a shows some differences between the strains resembling those seen in Fig. 1a. The predominating precipitates were formed by determinants of moderate electromobility containing mannan, but at such high concentrations that they were not totally retained by the hyperimmune anti-cell serum (see open arrows in Fig. 2a). As in Fig. 1a, contiguous spike precipitates were formed near the start position with *wt mann* but were absent with *m-10 mann.* In the intermediate gel with the patient serum, only one precipitate was discernible with each of these antigens. In contrast, Figs. 2b and 2c demonstrate molecules with the same mobility as that of a massive, homologous precipitate near the intermediate gel interface, confirming the predominant mannan character of the antigens. Quantitative differences between the *wt* and the *m-10* precipitates can be observed. Antigens, not bound by the Con A at this concentration (150 $\mu g/cm^2$), were precipitated by the anti-cell serum in the upper gel, indicating two precipitates with the *wt mann* versus one with the *m-10* (Fig. 2b). The extensions of precipitates through the intermediate gel into the upper gel in Fig. 2c imply the presence of reactive antibodies to the separated antigens in the anti-cell wall serum, but at low concentrations. In addition, the anti-cell wall serum

showed in the *m-10 mann* spectrum a double-peaked precipitate, not seen with the *wt mann.*

The material not bound by Con A affinity chormatography of the extracts, the *wt prot* and *m-10 prot* fractions, demonstrated, in the intermediate gel containing anti-cell wall serum, a larger number of precipitates with the *wt prot* than with the *m-10 prot* (10 and 6 precipitates, respectively). These lines were formed by antigens varying in concentration and electromobility, see Fig. 3a. Traces of carbohydrate in the protein fractions were detected at the site of the first dimension separation as elongated, very much reduced lines formed below the intermediate gel interface. Additional antigens were precipitated in the upper gel which contained hyperimmune anti-cell serum. In comparison, the differences found with the anti-cell wall serum were not seen when a patient's serum was incorporated in the intermediate gel (Fig. 3b). The *wt prot* and *m-10 prot* were unaffected by precipitins in the patients' sera and the total number of detected antigens, at least 15–20 precipitates, was formed in the upper gel or near the interface with the hyperimmune anti-cell serum; compare Figs. 3a and 3b.

Sera from 15 patients with culture-proven or suspected candidiasis were tested with crossed immunoelectrophoresis for their reactivity to the whole cell extracts from the *wt* and *m-10* strains. The results are summarized and grouped in Table I. From one to three, broad, deeply stained characteristic mannan precipitates with peaks at the moderate migration position were formed with the *wt* extract by all sera; nine of these sera formed more than one line and seven also precipitated the antigen(s) of very slow electromobility. With the *m-10* extract, eight of the sera from Group II patients formed one mannan precipitate at the moderate electromigration position, although four of these eight formed only an uncharacteristic, faint precipitate. None of the patients' sera revealed the slow migrating antigen with the *m-10* extract. The differences in the reactivity of the sera toward the *wt* and *m-10* strains were observed in the mannan components only at the moderate electromobility. Two to three precipitate lines were noted at other migration positions, without showing differences between the *wt* and the *m-10* extracts.

Discussion. In previous studies, *C. albicans* (*m-10*) was found to adhere *in vitro* less readily to fibrin–platelet matrices than the parental strain (*wt*) and infectivity in a rabbit model of endocarditis by *m-10* was also significantly lower than that with the parental strain (1). In addition, with the *wt* strain, surface mannan appeared to promote attachment of yeast cells to similar matrices (4). This observation led us initially to investigate purified mannan extracted by the Peat *et al.* procedure (18) from *wt* and *m-10* strains using several immunological methods. However, in comparing this purified polysaccharide, no obvious differences between the strains were noted. Therefore, parallel analyses of whole cell extracts from *wt* and *m-10* and two Con A fractioned antigens, consisting of soluble proteins (free of carbohydrates) and a heterologous complex of polysaccharides and glycoproteins (mannoprotein fraction), were performed with crossed immunoelectrophoresis. As counter reactants, a panel of sera from patients with proven or suspected candidiasis and rabbit hyperimmune sera raised against *wt* cells or a purified *wt* cell wall preparation were employed.

Whole cell extracts of the *wt* and *m-10* strains contained at least two cell wall antigens with moderate electromobility forming broad, deeply stained precipitates with sera from infected rabbits which resembled in character, electromigration, and number the polysaccharide complexes described by Axelsen (15). When a mannoprotein fraction with high carbohydrate content from a Con A affinity column was analyzed with hyperimmune anti-wt cell serum, the precipitation spectra contained a larger number of peaks, with the predominating precipitates strikingly increased in concentration, but otherwise resembling the aforementioned extract patterns. The mannosylated character of these antigens was tentatively confirmed by retardation of or binding to Con A (150 $\mu g/cm^2$) in the intermediate gel as demonstrated by Syverson and Buckley (19). In other experiments with a higher concentration of Con A (250 $\mu g/cm^2$), the separated antigen components were then completely retained in the intermediate gel. The similarity among homologous precipitates observed with Con A or anti-cell wall in the intermediate gels (compare Figs. 2b and 2c) suggested a high content of precipitins specific

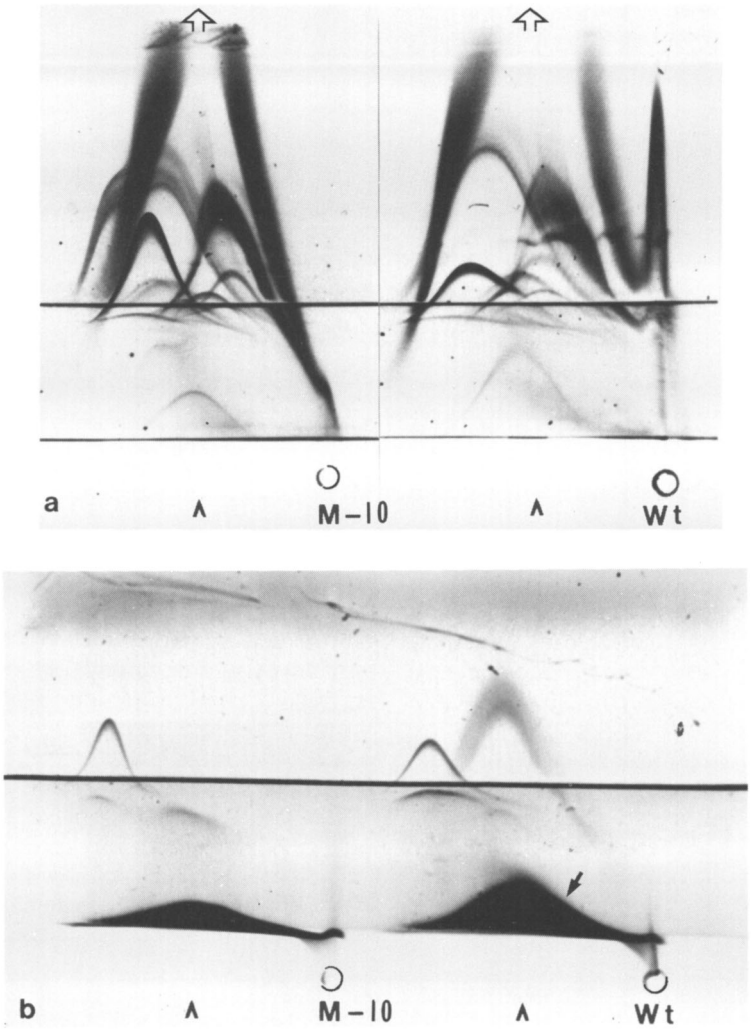

FIG. 2. Crossed immunoelectrophoresis of *m-10* and *wt mann* Con A fractions with a hyperimmune anti-*wt* cell serum in the upper gels. In (a), the patient serum in the intermediate gel retained one antigen from each fraction. Note the broad, intensely stained precipitates extending far into the upper gels (arrows). These lines coalesced again with spikes (arrow) near the start basin of *wt mann* but not *m-10 mann*. Antigenemia is suggested by the horizontal lines. The intermediate gels in (b) and (c) contain Con A and anti-*wt* cell wall serum, respectively. Note the similarity of the homologous stained precipitates near the borders of the intermediate gels extending to or below the start basins in the *wt mann* spectra (see arrows). The other precipitates may represent mannosylated proteins. The unique line in (c) with *m-10 mann* is indicated by an arrow.

to the mannosylated cell wall antigens in the anti-cell wall sera obtained by extended immunization. The separation of such complexes by crossed immunoelectrophoresis (with different reactants in the intermediate gels) may be a useful approach for the characterization of mannoprotein complexes, since SDS–PAGE analyses are often inadequate in resolving high-molecular-weight mannoproteins with high oligosaccharide content. The use of Tris–borate buffer for more extended electroseparation of the mannosylated antigens may be useful in future investigations.

Tests with patients' sera showed qualitative differences in reactivity to the cell extracts of the two strains. While all 15 sera recognized

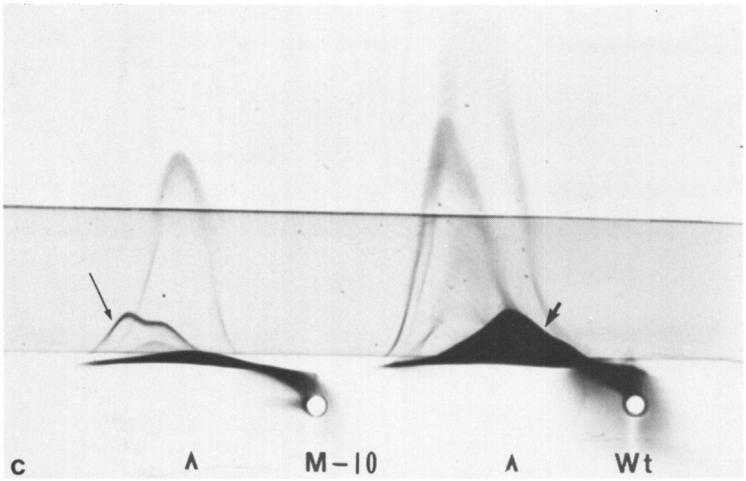

FIG. 2—*Continued.*

one to three epitopes at moderate electromobility in the *wt* extract, as mentioned by others (17, 20), with the *m-10* extract, only 4 of the Group II and none of the Group I sera developed typical mannoprotein precipitates. These observations indicate diversity in precipitin content of the patients' sera toward cell wall components of the two strains, thus, suggesting antigenic differences. Differences were also noted in regard to the spike-shaped precipitate formed in the second dimension near the site of the start basin. This line(s), seen with about 50% of the sera, was usually contiguous with a *wt mannan* precipitate and was never found in *m-10* patterns. It is possible that the molecules forming the spikes were nearly stationary in the first dimension, due to their charge or size. In the second dimension, upon combination with counter-migrating antibodies, their neutral charge was compensated permitting anodic migration of the antigen–antibody soluble complexes. Another explanation might be differences in the structure of the cell wall polysaccharide molecules, i.e., that the *wt* unlike the *m-10* has a special epitope(s). In any case, the "spike" was detected only in the *wt* preparations by using antisera against live cells of *C. albicans* (rabbits or patients).

The association of the spike-formed antigens with the cell wall is seen in Fig. 2c, where a diffuse precipitate may be seen around and cathodic to the *wt mann* basin, probably affected by the anti-cell wall serum in the intermediate gel. These precipitins, at high concentrations in the anti-cell wall sera, may differ in affinity and/or charge from those in the serum from infected rabbits, as illustrated in Fig. 1a. The anti-cell wall serum also formed a double-peaked precipitate with rapidly migrating antigens (arrow in Fig. 2c) in the *m-10 mann* preparation but not with those in the *wt mann.* It is possible that in *m-10,* posttranslational modifications (glycosylation) are different from those of *wt* cells, resulting in an epitope with variations in size or charge but which are otherwise similar to *wt;* these variant molecules would be recognized by patient sera as different from *wt.* Ongoing studies with DEAE fractionations and SDS–PAGE of *wt* and *m-10* extracts lend support to differentiating antigen(s) with unlike charge and molecular size.

Comparison of the soluble protein fractions, obtained by Con A chromatography of the *wt* and *m-10* extracts, showed less variation. While no substantial differences could be detected in the precipitation patterns of the *wt prot* and *m-10* with either hyperimmune rabbit anti-cell serum or the tested patients' sera, in comparison, the rabbit anti-cell wall serum precipitated more antigens in the *wt prot* than in the *m-10 prot.* Since the immune serum was prepared against *wt,* it appears that additional immunoprecipitates may be associated with antigenic determinants of the cell wall of the *wt* strain which are not detectable

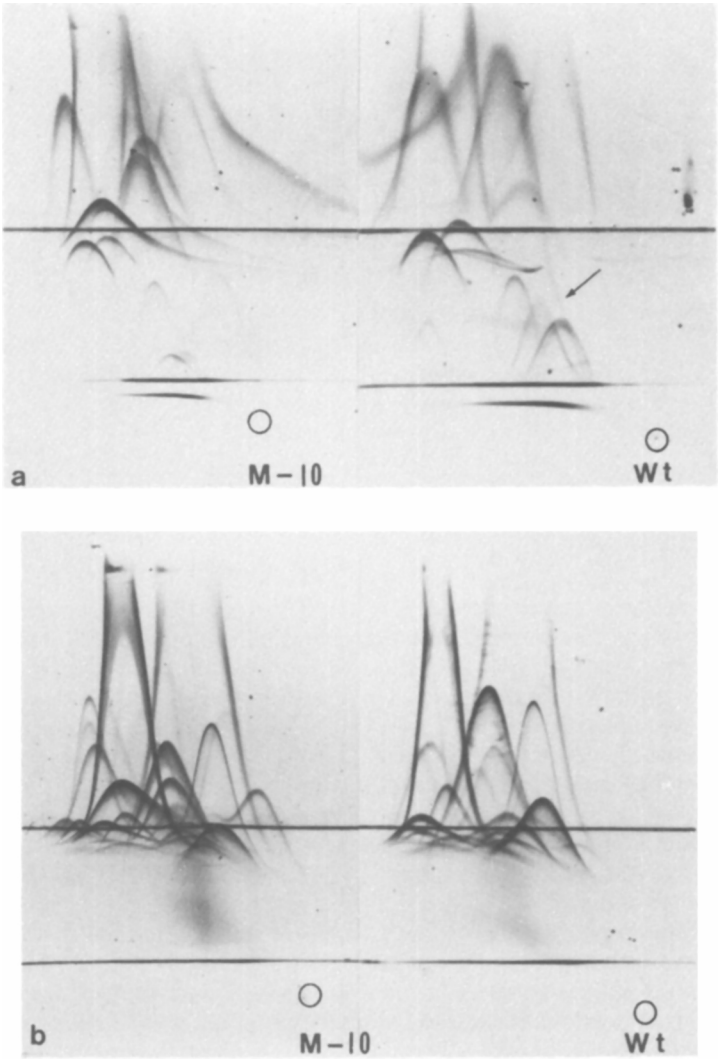

FIG. 3. Crossed immunoelectrophoresis of *m-10 prot* and *wt prot* Con A fractions with hyperimmune anti-*wt* cell serum in the upper gels and with anti-*wt* cell wall serum and serum from a Group II patient in the intermediate gels (a) and (b), respectively. Note the increase in the number of peaks for the *wt prot* (arrows) as compared to *m-10 prot* in the intermediate gel (a). No precipitins in the patient serum were clearly revealed by these protein fractions (b).

in the avirulent strain. Using Con A in the intermediate gel, no precipitate lines were detected when either *wt* or *m-10* protein was used as antigen confirming their nonglycosylated character. Only small traces of carbohydrate were again precipitated by Con A as elongated lines (data not presented).

Aside from the antigenic differences we observed between *wt* and *m-10*, our data clearly indicate that nonglycosylated proteins can be found in the cell wall of *C. albicans*. Chaffin and Stocco (21) showed that SDS extracts of purified cell wall contained a number of proteins which when analyzed by SDS–PAGE appeared to be nonglycosylated. In the present study, we used an immunological approach to substantiate the data of Chaffin and Stocco and have shown a number of such wall proteins (see Fig. 3a) using antisera made specifically against purified cell wall. The functions of these proteins remain to be established.

In conclusion, these preliminary results in-

TABLE I. CROSSED IMMUNOELECTROPHORETIC ANALYSES OF SERA FROM PATIENTS USING CELL EXTRACTS OF THE *wt* OR *m-10* STRAINS: REACTIVITY TO THE MANNAN COMPONENTS

Patients' sera			No. of mannan precipitates			
			wt extract Ag electromobility		*m-10* extract Ag electromobility	
Group[a]	Serum	Titer	Slow	Moderate	Slow	Moderate
I	B 277	1:16	2	2	0	0
	B 12	1:64	1	2	0	0
	B 294	1:32	0	1	0	0
	B 299	1:16	0	2	0	0
II	A 4677	1:8	0	1	0	0
	A 4657	1:16	0	1	0	0
	A 4567	1:8	1	3	0	0
	A 4684	1:16	0	2	0	(1)[b]
	A 4641	1:32	0	1	0	(1)
	A 4658	1:16	0	1	0	(1)
	A 4587	1:16	1	3	0	(1)
	A 4674	1:8	1	2	0	1
	A 4679	1:16	1	2	0	1
	A 4586	1:16	1	2	0	1
	A 4685	1:64	0	1	0	1
Total no. of reactive sera			7	15	0	8 (4)

[a] I. Culture-proven systemic candidiasis; antibody titer with latex agglutination.
[b] II. Suspected candidiasis; antibody titer with counter immunoelectrophoresis. ( ) indicates a faint, uncharacteristic line.

dicate that antigenic differences exist between the wild type, virulent *C. albicans* and the avirulent, derived strain. The findings also show that at least some of these *wt*-specific antigens are probably associated with the cell surface (cell wall). We are currently separating the mannoprotein complexes by ion-exchange chromatography and subjecting the fractionated antigens to Western blotting with polyvalent and monoclonal antibodies to define the specific *wt* immunodeterminants not detected in the mutant strain. Whether the *wt*-specific cell-surface antigens are associated with adherence and infectivity remains to be proven in more extensive investigations.

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1. Calderone RA, Cihlar RL, Dong-Soo Lee D, Hoberg K, Scheld WM. Yeast adhesion in the pathogenesis of endocarditis due to *Candida albicans:* Studies with adherence negative mutants. J Infect Dis **152:**710–715, 1985.
2. Scheld WM, Calderone RA, Alliegro GM, Sande MA. Yeast adherence in the pathogenesis of *Candida* endocarditis. Proc Soc Exp Biol Med **168:**208–213, 1981.
3. Maisch P, Calderone RA. Adherence of *Candida albicans* to a fibrin–platelet matrix formed *in vitro.* Infect Immun **27:**650–656, 1980.
4. Maisch P, Calderone RA. Role of surface mannan in the adherence of *Candida albicans* to fibrin–platelet clots formed *in vitro.* Infect Immun **32:**92–97, 1981.
5. Lee JC, King RD. Characterization of *Candida albicans* adherence to human vaginal epithelial cells *in vitro.* Infect Immun **41:**1024–1030, 1983.
6. Sandin RL, Rogers AL, Patterson RJ, Beneke ES. Evidence for mannose-mediated adherence of *Candida albicans* to human buccal cells *in vitro.* Infect Immun **35:**79–85, 1982.
7. Segal E, Lehrer N, Ofek I. Adherence of *Candida albicans* to human vaginal epithelial cells: Inhibition by amino sugars. Exp Cell Biol **50:**13–17, 1982.
8. Manning M, Mitchell TG. Analysis of cytoplasmic antigens of the yeast and mycelial phases of *Candida albicans* by two dimensional electrophoresis. Infect Immun **30:**484–495, 1980.
9. Hoberg K, Cihlar RL, Calderone RA. Characterization of cerulenin-resistant mutants of *Candida albicans.* Infect Immun **51:**102–109, 1986.
10. Ellsworth JH, Reiss E, Bradley RL, Chmel H, Armstrong D. Comparative serological and cutaneous

reactivity of candidial cytoplasmic proteins and mannan separated by affinity for Concanavalin A. J Clin Microb **5**:91–99, 1977.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
12. Hodge JE, Hofreiter BT. Determination of reducing sugars and carbohydrates. In: Wistler RL, Wolfrom ML, Eds., Methods in Carbohydrate Chemistry. New York: Academic Press, Vol **1**:pp380–394, 1962.
13. Reiss E, Stone SH, Hasenclever HF. Serological and cellular immune activity of peptidoglucomannan fractions of *Candida albicans* cell walls. Infect Immun **9**:881–890, 1974.
14. Laurell CB. Antigen–antibody crossed electrophoresis. Anal Biochem **10**:358–361, 1965.
15. Axelsen NH. Quantitative immunoelectrophoretic methods as tools for a polyvalent approach to standardization in the immunochemistry of *Candida albicans*. Infect Immun **7**:949–960, 1973.
16. Axelsen NH (Ed). Intermediate gel immunoelectrophoresis. Scand J Immunol **17**(Suppl. 10):141–149, 1983.
17. Axelsen NH, Kirkpatrick CH, Buckley RH. Precipitins to *Candida albicans* in chronic mucocutaneous candidiasis studied by crossed immunoelectrophoresis with intermediate gel. Correlation with clinical and immunological findings. Clin Exp Immunol **17**:385–394, 1974.
18. Peat S, Whelan WJ, Edwards TE. Polysaccharides of baker's yeast. Part IV. Mannan. J Chem Soc (London) **1**:29–34, 1961.
19. Syverson RE, Buckley HR. Cell wall antigens in soluble cytoplasmic extracts of *Candida albicans* as demonstrated by crossed-affimmunoelectrophoresis with concanavalin A. J Immunol Methods **18**:149–156, 1977.
20. Glew RH, Buckley HR, Rosen HM, Moellering RC, Fisher JE. Serologic test in the diagnosis of systemic candidiasis. Amer J Med **64**:586–591, 1978.
21. Chaffin WL, Stocco DM. Cell wall proteins of *Candida albicans*. Canad J Microb **29**:1438–1444, 1983.

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