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Production and Characterization of Agglutinating Monoclonal Antibodies against Predominant Antigenic Factors for *Candida albicans*

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Two clones, CA4-2 and CA5-4, which produced agglutinating monoclonal immunoglobulin M (IgM) antibodies (MAbs) against mannan antigens of *Candida albicans* serotype A, were established. The specificity of each MAb was determined by slide agglutination tests for cross-reactivity patterns against the homologous and six other strains of *Candida* and a strain of *Torulopsis*: *C. albicans* serotype B, *C. tropicalis*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, and *Torulopsis glabrata*. The MAb produced by CA4-2 reacted with the homologous, *C. tropicalis*, and *T. glabrata* strains, whereas the MAb produced by CA5-4 reacted with the homologous, *C. albicans* serotype B, and *C. tropicalis* strains. These results are consistent with results obtained by comparative experiments with several strains of each serotype or species. Specificity of these two MAbs by agglutination was also consistent with the cross-reactivity patterns demonstrated by indirect immunofluorescence staining. The competitive binding experiments by immunofluorescence staining with two MAbs and polyclonal factor sera (PAb factors) 5 and 6 suggested that the MAb from clone CA4-2 did not completely correspond to PAb factor 6 and that the MAb from CA5-4 was distinct from PAb factor 5 in its manner of binding to determinants (the latter was designated 5b). Cross-reactivity patterns, however, furnished evidence that these two MAbs could replace the known PAb factors 6 and 5, respectively, as reagents for aid in the identification of the strains of *C. albicans* and their serotypes.

Tsuchiya et al. (23) determined the antigenic patterns of the medically important yeasts by cross-absorption experiments and proposed antigenic formulas for their serological classification. They produced and applied monospecific or absorbed antisera to identify the members of the genus *Candida* based on their antigenic formulas. Subsequently Fukazawa et al. (6) and Fukazawa and Tsuchiya (7) designed a slide agglutination scheme which uses factor sera, whereby it is possible to make rapid and accurate identification of medically important *Candida* species including *Candida albicans* serotypes A and B (5, 11). However, because there exist complicating common antigens among these *Candida* species, cross-absorption for the preparation of factor sera is often accompanied by a concomitant decrease in their antibody titers.

To overcome this problem, preparation of monoclonal antibody (MAb), which is directed against a single antigenic determinant, is required in place of conventional factor sera. Although many MAbs against cell surface antigens of a variety of microorganisms (10, 12, 17, 19) have been reported, there are only a few reports on MAbs against *Candida* species (3, 18). Brawner and Cutler (3) have prepared agglutinating MAb against *C. albicans* which reacts with *C. albicans* serotype A, *C. tropicalis*, and *Torulopsis glabrata*. However, they used this antibody to determine the time course of antigen synthesis and heterogeneity of antigens in the population rather than for serological identification.

Therefore, as a first step in establishing a system for serological identification of *Candida* species by using MAbs, we attempted to prepare agglutinating MAbs against cells of *C. albicans* serotype A. In this paper, we report on the specificity of such MAbs from two successfully obtained clones and their significance in the serological identification of *C. albicans* including its serotypes.

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MATERIALS AND METHODS

Strains. The standard strains used in this study and their antigenic formulas are listed in Table 1. All strains were stock cultures maintained in our laboratory. The strains used for comparative experiments were stock cultures maintained in Iatron Laboratories (Tokyo).

Extraction and purification of the polysaccharides. Yeast cells grown on modified Sabouraud glucose agar (2% glucose, 1% polypeptone, 0.5% yeast extract [Difco Laboratories, Detroit, Mich.], 1.5% agar) at 27°C for 48 h were harvested, washed three times with distilled water, heated at 100°C for 2 h, and centrifuged. After centrifugation, the mannans were extracted with alkali (4) and purified as a copper complex (9). Alkali-extracted mannans of several *Candida* species possess serotype-specific determinants (22).

Immunization. Six-week-old BALB/c mice (Charles River Japan Inc., Kanagawa, Japan) were intraperitoneally injected weekly with 0.2 ml of a heat-killed cell suspension (5×10^7 to 20×10^7 cells per ml) of *C. albicans* M1012 (serotype A). Antibody response was monitored by the quantitative slide agglutination test.

Hybridoma production. Murine myeloma cell line X63·Ag8·653 was kindly provided by K. Tasaka. The cells were cultured in RPMI 1640 medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 15% fetal bovine serum (M. A. Bioproducts, Walkersville, Md.). Cell fusion was carried out by the method of Galfre et al. (8). Briefly, 6×10^7 spleen cells of the hyperimmune mice 3 days after the last injection were fused with 3×10^7 myeloma cells with

TABLE 1. Antigenic formulas of *Candida albicans* and related species

Strain	Antigenic factors
C. albicans M1012 (serotype A)	1, 4, 5, 6
C. albicans M1445 (serotype B)	1, 4, 5, 13b
C. tropicalis M1017	1, 4, 5, 6
C. guilliermondii M1023	1, 4, 9
C. krusei M1005	1, 5[a], 11
C. parapsilosis M1015	1, 5[a], 13, 13b
C. pseudotropicalis M1004	1, 8
T. glabrata M4002	1, 4, 6, 34

[a] Occasionally negative, depending on the lot of factor sera.

50% polyethylene glycol 4000 (Merck & Co., Inc., Rahway, N.J.). The cell suspension in HAT medium (14) was seeded in microwell plates (No. 3072, Becton Dickinson Labware, Oxnard, Calif.) containing 1×10^4 peritoneal cells per well.

Culture supernatants were screened for antibody production by enzyme immunoassay (EIA) (13). Flat-bottomed MicroELISA plates (Immulon II; Dynatech, Plochingen, Federal Republic of Germany) which were coated for 2 h at 37°C with 100 μl of a 25 μg/ml solution of *C. albicans* M1012 cell wall mannan in 0.05 M carbonate buffer (pH 9.6) and blocked with 200 μl of a 1% gelatin solution in phosphate-buffered saline solution (PBS) were used for EIA. A mixture of peroxidase-labeled goat anti-mouse immunoglobulins (immunoglobulin A [IgA] plus IgG plus IgM; heavy plus light chain specific; Cooper Biomedical, Inc., West Chester, Pa.) was used as second antibody, and the binding of second antibody was determined by using *o*-phenylenediamine-hydrogen peroxide as substrate. The wells that were positive for antibody against the coating mannan antigen were detected visually. The antibody-producing hybridomas were subcloned twice by limiting dilution and were tested for antibody production directly by the slide agglutination reaction with heat-killed cells of *C. albicans* M1012. Ascites fluids obtained by injecting hybridomas (5×10^6) intraperitoneally into pristane-treated BALB/c mice were used for characterization of MAbs.

Slide agglutination test. (i) Quantitative slide agglutination test. Agglutinin titers were determined by the slide agglutination test. Briefly, 25 μl of serial twofold dilutions of the sample were made in a series of circles on a slide. The same volumes of heat-killed cell suspension (in physiological saline with 0.5% Formalin [0.18% formaldehyde]) adjusted to McFarland standard no. 10 (A_{560} = 2.4) were added, and the slide was rotated at 100 rpm for 10 min. The highest dilution showing agglutination within the 10-min rotation was defined as the titer of the sample in a quantitative slide agglutination test.

(ii) Rapid slide agglutination test. The appropriately diluted sample was mixed homogeneously with a sufficient amount of viable cells grown on Sabouraud glucose agar, and the agglutination within 1-min rotation was determined.

Immunofluorescence test. Indirect immunofluorescence staining (4) was carried out for the standard strain of each *Candida* species and for *T. glabrata*. Yeast cells fixed on the slide by heat were first reacted with a drop of 20-fold-diluted ascites fluid (titer 1:64) at room temperature for 45 min in a moist chamber. After the smear was washed with PBS it was reacted at room temperature for 45 min in a moist chamber with a drop of fluorescein isothiocyanate (FITC)-labeled rabbit anti-mouse immunoglobulin serum (Cedarlane Laboratories, Ontario, Canada) diluted 10-fold with PBS. After being washed with PBS, the slide was mounted in PBS containing 90% glycerol. The stained preparations were examined with a fluorescence microscope (BHS-RFK; Olympus Optical Co. Ltd., Tokyo).

Absorption test. Diluted ascites fluid (titer, 1:128) was mixed with an equal volume of packed heat-killed cells. The suspension was incubated for 2 h at 37°C and overnight at 4°C and centrifuged, and the agglutinin titer of the supernatant was determined by the quantitative slide agglutination reaction.

Competitive binding test. Smears of heat-killed cells of *C. albicans* M1012 were first incubated twice with rabbit polyclonal factor serum 6 (PAb factor 6) (24) at room temperature for 45 min, washed with PBS, and incubated with MAb CA4-2. After being washed with PBS, the smears were incubated with FITC-labeled anti-mouse immunoglobulin rabbit serum. Heat-killed cells of *C. albicans* M1445 were incubated with PAb factor 5 followed by MAb CA5-4 and were stained in the same manner. Conversely, smears of heat-killed cells of *C. albicans* M1012 or *C. albicans* M1445 were first incubated twice with MAb CA4-2 or MAb CA5-4, washed with PBS, and incubated again with PAb factor 6 or 5, respectively. After being washed with PBS, the smears were incubated with FITC-labeled anti-rabbit IgG sheep serum (Cooper Biomedical).

Determination of immunoglobulin class. (i) Gel filtration. Ascites fluids were fractionated by gel filtration on a Sephadex G-200 column (Pharmacia, Uppsala, Sweden) as described previously (6). The antibody fractions were titrated by the quantitative slide agglutination test.

(ii) 2-ME sensitivity. A 10-fold dilution of ascites fluid (100 μl) was treated with 0.1 M 2-mercaptoethanol (2-ME) for 2 h at 37°C (15). After the treatment, the agglutinin titer of the sample was determined as described above.

(iii) Immunodiffusion test. Ascites fluids and mouse μ-chain and each of the γ-chain-specific rabbit sera (Pel-Freeze Biochemicals, Rogers, Ariz.) were tested by immunodiffusion in 1% agarose (ME; FMC Corp., Marine Colloids Div., Rockland, Maine) in 0.02 M PBS. Holes (3 mm) in agarose plates (8 mm apart, center to center) in plastic dishes (35 by 10 mm; Becton Dickinson) were filled with ascites fluid or antisera. Reactions were read after 24 or 48 h of incubation at room temperature.

RESULTS

Immune responses of BALB/c mice to *C. albicans* heat-killed cells. Twenty BALB/c mice were injected weekly with heat-killed cells of *C. albicans* M1012, and their immune responses were monitored by the quantitative slide agglutination test. The timing and the rate of increase in the agglutinin titer of the immunized mice varied; 4 to 9 weeks were required to reach the maximum agglutinin titer (1:640 to 1:1,280) after the first injection. The agglutinin activity of the immune sera was 2-ME sensitive.

Hybridoma production. Spleen cells from a mouse that was hyperimmune to *C. albicans* M1012 were fused with myeloma cells. After cultivation in HAT medium for about 2 weeks, two wells were EIA positive with *C. albicans* M1012 mannan. Cells from these two wells were cloned twice separately by limiting dilution, and clones CA4-2 and CA5-4 were obtained. The supernatants of both clones agglutinated strongly with *C. albicans* M1012 cells. In three other experiments with the same conditions, a clone which produced agglutinating antibody was not obtained, although many EIA-positive clones were obtained.

Immunoglobulin class of MAbs. Ascites fluids from mice

TABLE 2. Titers in quantitative slide agglutination of ascites fluid from clones CA4-2 and CA5-4

Antigen	Agglutinin titer	
	CA4-2	CA5-4
C. albicans M1012	1:1,280	1:1,280
C. albicans M1445	<1:2	1:1,280
C. tropicalis M1017	1:640	1:1,280
C. guilliermondii M1023	<1:2	<1:2
C. krusei M1005	<1:2	<1:2
C. parapsilosis M1015	<1:2	<1:2
C. pseudotropicalis M1004	<1:2	<1:2
T. glabrata M4002	1:640	<1:2

inoculated with CA4-2 and CA5-4 were pooled and applied to a Sephadex column. The agglutinin activities of both samples were found exclusively in the first peak (void volume). The agglutinin titers of both ascites fluids were greatly reduced (<1:20) by the 2-ME treatment. Moreover, the ascites fluids formed precipitin bands with μ-chain-specific antiserum but not with γ-chain-specific antiserum in an immunodiffusion test. These results indicate that the class of immunoglobulins produced by these clones is IgM.

Specificity of MAbs. To determine the specificity of the MAbs produced by clones CA4-2 and CA5-4, we examined these MAbs for cross-reactivity with seven strains from six species of *Candida* and from *T. glabrata* by a slide agglutination test and immunofluorescence staining (Table 2 and Fig. 1). The ascites fluid from CA4-2 showed agglutinin titers of 1:1,280, 1:640, and 1:640 against *C. albicans* M1012 (serotype A), *C. tropicalis* M1017, and *T. glabrata* M4002, respectively, whereas it showed no detectable reaction with the other *Candida* strains under the conditions used (1:2 dilution). In contrast, the ascites fluid from CA5-4 showed an agglutinin titer of 1:1,280 against *C. albicans* M1012, *C. albicans* M1445 (serotype B), and *C. tropicalis* M1017, whereas it showed no detectable reaction with the other strains. Indirect immunofluorescence staining by using the heat-killed cells of each *Candida* and *Torulopsis* strain, the ascites fluid, and FITC-labeled rabbit anti-mouse immunoglobulin serum was also performed. All the strains that were agglutinated by the ascites fluid from CA4-2 or CA5-4 were clearly stained by immunofluorescence, although cells of *C. krusei* M1005 were weakly stained with the CA5-4 antibody in spite of the absence of detectable agglutination with the same antibody. Considering the antigenic formulas of these *Candida* and *Torulopsis* strains (Table 1) reported by Fukazawa et al. (5), it was suggested that the specificities of MAbs produced by clones CA4-2 and CA5-4 are comparable to PAb factor 6 and 5, respectively.

To compare the specificity of MAbs and PAbs, cross-absorption experiments were performed (Table 3). Absorption of PAb factor 6 and MAb CA4-2 with *C. albicans* serotype B did not reduce the agglutinin titers to *C. albicans* serotype A, *C. tropicalis*, and *T. glabrata*. Conversely, absorption of PAb factor 6 and MAb CA4-2 with *T. glabrata* eliminated reactivity with *C. albicans* serotype A and *C. tropicalis*. Similarly, absorption of PAb factor 5 and MAb CA5-4 with *T. glabrata* did not reduce agglutinin titers to *C.*

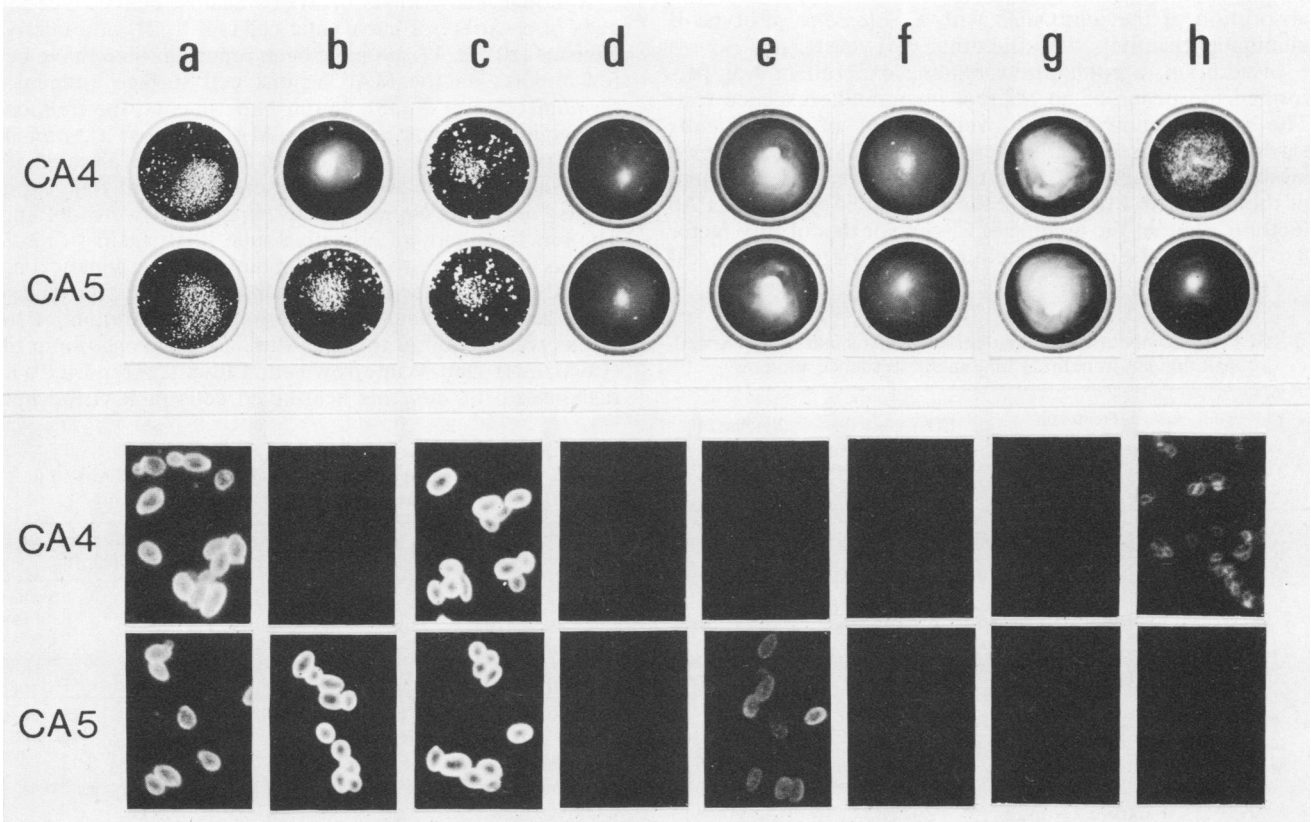

FIG. 1. Slide agglutination test (upper panel) and indirect immunofluorescence staining (lower panel) of *C. albicans* and related species with MAbs CA4-2 and CA5-4. A 20-fold dilution of each ascites fluid (original titer, 1:1,280) was used for the tests. (a) *C. albicans* M1012; (b) *C. albicans* M1445; (c) *C. tropicalis* M1017; (d) *C. guilliermondii* M1023; (e) *C. krusei* M1005; (f) *C. parapsilosis* M1015; (g) *C. pseudotropicalis* M1004; (h) *T. glabrata* M4002. CA4 and CA5 represent CA4-2 and CA5-4, respectively.

TABLE 3. Absorption of MAbs CA4-2 and CA5-4 and PAbs 5 and 6 with *C. albicans* serotype B and *T. glabrata*

Antibody and absorbing strain	Agglutinin titer to:			
	C. albicans serotype A	*C. albicans* serotype B	*C. tropicalis*	*T. glabrata*
CA4-2				
None	1:128	<1:2	1:64	1:64
C. albicans serotype B	1:128	<1:2	1:64	1:64
T. glabrata	<1:2	<1:2	<1:2	<1:2
Factor 6				
None	1:64	<1:2	1:32	1:16
C. albicans serotype B	1:64	<1:2	1:32	1:16
T. glabrata	<1:2	<1:2	<1:2	<1:2
CA5-4				
None	1:128	1:128	1:128	<1:2
C. albicans serotype B	<1:2	<1:2	<1:2	<1:2
T. glabrata	1:128	1:128	1:128	<1:2
Factor 5				
None	1:32	1:32	1:32	<1:2
C. albicans serotype B	<1:2	<1:2	<1:2	<1:2
T. glabrata	1:32	1:32	1:32	<1:2

albicans serotypes A and B and *C. tropicalis*, although absorption of the antibodies with *C. albicans* serotype B eliminated reactivity with the other two yeasts.

In addition, a competitive binding experiment was performed by means of an indirect immunofluorescence test. The results showed that pretreatment of *C. albicans* serotype A cells with a sufficient amount of PAb factor 6 inhibited the binding of MAb CA4-2, whereas pretreatment of the cells with MAb CA4-2 did not inhibit binding of PAb factor 6. Neither the binding of CA5-4 nor that of PAb factor 5 to *C. albicans* serotype B cells (selected for the binding test because of their strong reactivity to MAb CA5-4 in immunofluorescence staining) was inhibited by pretreatment of the cells with either PAb factor 5 or CA5-4, respectively (Table 4). These results suggest that MAb CA4-2 shares a part of the binding sites of determinants on yeast cell walls with PAb factor 6, and that CA5-4 does not share any binding sites with PAb factor 5.

TABLE 4. Competitive binding between PAbs and MAbs to cell wall antigen in indirect immunofluorescence staining

Antigen	Treatment		Stained with FITC-labeled antiserum against immunoglobulins of	Binding of FITC-labeled antiserum[a]
	1st	2nd		
C. albicans serotype A, M1012	CA4-2	None	Mouse	+
	Factor 6	None	Mouse	−
	Factor 6	CA4-2	Mouse	−
	Factor 6[b]	None	Rabbit	+
	CA4-2	None	Rabbit	−
	CA4-2	Factor 6	Rabbit	+
C. albicans serotype B, M1445	CA5-4	None	Mouse	+
	Factor 5	None	Mouse	−
	Factor 5	CA5-4	Mouse	+
	Factor 5	None	Rabbit	+
	CA5-4	None	Rabbit	−
	CA5-4	Factor 5	Rabbit	+

[a] +, Binding; −, no binding.
[b] PAb factors were prepared from rabbit antiserum.

Application of MAbs to identification of *Candida* species. As an attempt to apply the ascites fluids from these clones to serological identification of *Candida* species, comparative experiments by rapid slide agglutination were performed with about 100 strains belonging to the six *Candida* species, including *C. albicans* serotypes A and B, and *T. glabrata* (maintained in Iatron Laboratories) (Table 5). In the 20-fold-diluted ascites fluid (titer, 1:64) from CA4-2, all the strains of *C. albicans* serotype A, *T. glabrata*, and 11 of 12 strains of *C. tropicalis* tested exhibited positive reactions, whereas none of the strains of the other five species exhibited positive reactions. On the other hand, in the ascites fluid (titer, 1:64) from CA5-4, all the strains of *C. albicans* serotypes A and B and *C. tropicalis* exhibited positive reactions, whereas none of the strains of the other five species exhibited positive reactions, although half of the *C. guilliermondii* strains reacted weakly at this concentration. Therefore, these results suggest the possibility that the MAbs produced by clones CA4-2 and CA5-4 could replace PAb factors 6 and 5, respectively, as reagents for serological identification of the strains of *C. albicans* and their serotypes.

DISCUSSION

Although many kinds of MAbs against cell surface antigens of a variety of eucaryotic cells (1, 8, 21) and microorganisms (10, 12, 17, 19) have been reported, there have been few reports on the MAb against cell surface antigens of *Candida* species (3, 18). In our experiments, the frequency of obtaining two agglutinating MAbs against *C. albicans* serotype A can be summarized as follows: although 876 hybridoma-positive wells were screened from four experiments, only two clones in one experiment were obtained. The low frequency of obtaining such MAb against *Candida* species might be attributable mainly to the genetic background of the lower immune response of BALB/c mice to *Candida* cells. Under our experimental conditions, 4 to 9 weeks were required to reach the maximum agglutinin titer (1:640 to 1:1,280). Winterrowd and Cutler (25) reported a low response to *C. albicans* heat-killed cells in BALB/c mice.

TABLE 5. Slide agglutination test with two MAbs in a comparative experiment

Species	No. of strains tested	Slide agglutination (no. of strains) with			
		Ascites fluid from CA4-2		Ascites fluid from CA5-4	
		+	−	+	−
C. albicans serotype A	16	16	0	16	0
C. albicans serotype B	14	0	14	14	0
C. tropicalis	12	11	1	12	0
C. guilliermondii	8	0	8	5[a]	3
C. krusei	15	0	15	0	15
C. parapsilosis	6	0	6	0	6
C. pseudotropicalis	9	0	9	0	9
T. glabrata	12	12	0	0	12

[a] Weakly positive.

Reiss et al. (18) also reported that only about one-third of the mice of two inbred strains and one-half of those of an outbred strain produced antibodies in response to *C. tropicalis* cell wall antigen and that the responding mice produced IgM, not IgG, suggesting that mannan behaves as a T-cell-independent antigen with poor anamnestic response as detected by indirect EIA.

The difficulty might be also related to the time of obtaining spleen cells from hyperimmune mice. A sharply increased agglutinin titer seemed to be essential to obtain the hybridomas producing the antibodies, since our many attempts using spleen cells from the mice which showed gradually increased agglutinin titer were unsuccessful.

The two MAbs produced by our clones CA4-2 and CA5-4 strongly agglutinated *C. albicans* serotype A, *C. tropicalis*, and *T. glabrata*, and *C. albicans* serotypes A and B and *C. tropicalis*, respectively. It is suggested that although mice were immunized with heat-killed cells of *C. albicans*, these MAbs were directed against the cell wall mannan of *C. albicans*, because they were selected first by EIA for partially purified mannan (protein content was less than 1%). Cross-reaction patterns demonstrated by slide agglutination corresponded fairly well to those obtained by immunofluorescence. However, immunofluorescence staining seemed to be slightly more sensitive than the agglutination reaction, since *C. krusei*, which was not agglutinated by the antibodies from CA5-4, was weakly stained by this antibody. Cross-absorption experiments showed that MAbs CA4-2 and CA5-4 were absorbed by the yeast cells possessing antigenic factor 6 and factor 5, respectively, but not by yeast cells without antigenic factor 6 and 5, respectively. Competitive binding experiments using two MAbs and PAb factors 5 and 6 suggested that MAb CA4-2 shared a part of the binding sites of determinants on yeast cell walls with PAb factor 6 and that CA5-4 did not share any binding sites with PAb factor 5. These results indicate that MAb produced by CA4-2 corresponds to PAb factor 6 and that antibody from CA5-4 is closely related to PAb factor 5 (23, 24) in their specificities, although the manner of recognition in MAb CA4-2 and CA5-4 is not the same as in PAbs 6 and 5, respectively. Further study of the MAbs with different recognition manners with the same specificity remains to be conducted.

Brawner and Cutler (3) have also reported an agglutinating IgM MAb which is reactive with *C. albicans*, *C. tropicalis*, and *T. glabrata*. From the antigenic formulas presented by Tsuchiya et al. (24) and also from our results, their MAb may be similar to our antibody from CA4-2 corresponding to antibody factor 6. Reiss et al. (18) produced MAbs against *C. tropicalis*, one IgM and one IgG that bound to mannans of *C. albicans* serotypes A and B, *C. tropicalis*, and two IgM antibodies specific for serotype A. The specificity of the former two MAbs and that of the latter two MAbs is assumed to resemble that of our MAb from CA5-4 and MAb from CA4-2, respectively, although the agglutinating ability and cross-reaction patterns of the clones for the six *Candida* species, and especially for *T. glabrata*, are not clear (18).

A commercial kit, Candida Check, has been produced by Iatron Laboratories and is based on the antigenic formulas proposed by Fukazawa et al. (6, 7). The kit consists of antisera for antigens 1, 4, 5, 6, 8, 9, 11, 13, 13b, and 34, and is widely used in clinical laboratories in Japan. In addition, a rapid biochemical test, the sucrose disk test, is recommended because strains of *C. albicans* serotype A and *C. tropicalis* are serologically indistinguishable. The test discriminates between *C. albicans* serotype A (negative reaction) and *C. tropicalis* (positive reaction). Shinoda et al. (20) compared Candida Check, the API-20C kit, and the conventional modified Wickerham technique in terms of rapid and accurate identification of medically important *Candida* species, and revealed that, for the *Candida* species tested, Candida Check gave a 95% accurate result.

TABLE 6. Revised antigenic formulas for six species of *Candida* and for *T. glabrata*

Species and serotype	Antigenic formula
C. albicans A	1, 4, **5b**,[a] **6**
C. albicans B	1, 4, **5b**
C. tropicalis	1, 4, **5b**, **6**
C. guilliermondii	1, 4, 9
C. krusei	1, 11
C. parapsilosis	1, 13
C. pseudotropicalis	1, 8
T. glabrata	1, 4, **6**, 34

[a] Boldface type indicates antigenic factors deduced from MAbs.

The factor sera used in Candida Check were sometimes claimed to be of higher titer. However, their preparation by one or two absorption steps is often accompanied by a concomitant decrease in agglutinins. Surprisingly, our two MAbs demonstrated agglutinin titers of 1:1,280 as the factor sera. As already mentioned, MAb produced by CA4-2 is recognized to correspond to factor 6. The other MAb, produced by CA5-4, is closely related to factor 5 in its agglutination pattern with the exception that this MAb does not agglutinate *C. krusei* and *C. parapsilosis* (Table 5). It is very interesting and significant to obtain an MAb that is strongly reactive with the strains of *C. albicans*, including serotypes A and B. Moreover, comparative experiments using several strains other than standard strains revealed that these two MAbs could be applicable to a diagnostic system. Therefore, we propose that these MAbs be used as new factor 5 and 6 in place of the old factors. Improved antigenic formulas based in part on the two new MAbs for identifying the members of medically important yeasts are shown in Table 6. In addition, factor 13b (Table 1), which has been shown to be variable in agglutinating *C. albicans* serotype B strains, could be deleted from the diagnostic antigenic formula, because (i) a high-titered new factor serum (designated factor 5b) specific for all the members of *C. albicans* has been obtained as MAb and (ii) *C. stellatoidea* has been reduced to synonymy with *C. albicans* in spite of its ability to assimilate sucrose (16). Further studies for obtaining MAbs directed against other important antigenic factors for a serological identification system are in progress in our laboratories.

Serological specificity of the yeast species is dependent upon the chemical structure of their cell wall mannans, which possess antigenic determinants (2). Regarding the chemical structure of antigenic factor 6, Suzuki and Fukazawa (22) reported that the dominant determinant of antigenic factor 6 is a mannohexaose consisting of one terminal $\alpha(1\text{-}3)$ linkage and four $\alpha(1\text{-}2)$ linkages and might be bound, via the $\beta(1\text{-}6)$ linkage, to C-6 of the mannose residues involved in the oligosaccharide side chains of serotype A mannan. Our two MAbs may be useful also as reagents for revealing the immunochemical structures of specific determinants of *C. albicans* cell wall mannans.

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