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Response and Specificity of Antibodies for *Candida albicans*

YOSHIMURA FUKAZAWA, TAKAKO SHINODA, AND TAKESHI TSUCHIYA
Department of Bacteriology, School of Medicine, Juntendo University, Tokyo, Japan

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Rabbit antibodies for *Candida albicans*, reacting in agglutination and fluorescent-antibody reactions, were present in both IgM and IgG protein fractions. The two types of immune globulins were separated from ammonium sulfate-precipitated γ-globulin either by filtration through a column of Sephadex G-200 or by diethylaminoethyl column chromatography performed by stepwise elution with various concentrations of sodium chloride. In the fluorescent-antibody test, initial separation of the IgG fraction, prior to its conjugation with dye, proved to be essential for the high specificity of this reaction. Investigation of the specificities of the two types of antibodies revealed that the IgG was highly specific, whereas the IgM was not very specific. Each antigen fraction, extracted by various methods, demonstrated its own characteristic antibody response. Only the IgG fraction yielded serotype-specific antibody useful for detection of a serotype of *C. albicans* in agglutination and fluorescent-antibody tests. The results indicate the importance of IgG for specific serological reactions with the *Candida* species.

In recent years, several investigators have reported on the serological relationships among several species of the genus *Candida* (2, 10, 18, 22, 24, 25). On the basis of our studies of the antigenic structures of many species of yeasts, we proposed a new system for their classification (26). More recently, it has been reported that antibody proteins with an electrophoretic mobility of γ-globulin consist of heterogeneous molecules. The antibody proteins in the serum of man (23), rabbits (20), and a number of other animals (6) are the 7*S* and 19*S* fractions of γ-globulin. Antibodies in these fractions may show degrees of reactivity different from those of antibodies in whole serum. The rapidly advanced methodology in immunochemistry demands a new approach to the serological methods used for classification and identification of microorganisms.

In recent studies of antibody activities in fractions of rabbit antisera for *Shigella flexneri* 1b and 2a, we have found that the specificities of the two types of antibodies differ from each other (27). The IgG demonstrated a high specificity and the IgM had a low specificity. It was considered worthwhile to apply these immunochemical findings further, in order to analyze the antibodies of yeast antigens reactive in agglutination and fluorescent-antibody tests, and to reexamine the preparation of factor antibodies based upon the newly described antigenic structures.

The present paper deals with the fractionation of antibody proteins and the specificity and response of two types of antibodies in anti-*C. albicans* serum.

MATERIALS AND METHODS

Strains used. The strain of *C. albicans* and the other yeasts used as antigens for immunization of rabbits and for agglutination tests are listed in Table 1, together with the antigenic structure of each species and the sources from which they were obtained.

Preparation of antisera. Antiserum for *C. albicans* was prepared by immunizing rabbits with heat-killed cells according to a method previously described (24). Several rabbits were immunized, and the harvested antisera were pooled to minimize the possibility of variation in individual response. Antisera for each crude extracted antigen were prepared as follows. Each 5 mg of crude antigen (1 mg of crude protein) was dissolved in 2 ml of distilled water and then mixed with an equal volume of Freund's complete adjuvant. Rabbits were injected once into the footpad with. Each antigen. The rabbits were bled from their ear veins every week after injection, and the serum pooled every week was tested for mode of antibody production.

Preparation of antibody fractions. The γ-globulin fractions were obtained by salting-out with half-saturated ammonium sulfate, after which some were fractionated by the gel filtration method (13). Sephadex G-200 (Pharmacia, Uppsala, Sweden) was used for gel filtration. The gel was equilibrated with 0.2 M tris(hydroxymethyl)aminomethane (Tris)-0.5 M gly-

TABLE 1. *Antigenic structures and sources of Candida and Cryptococcus strains*

Serogroup	Species	Source[a]	Antigenic structures[b]
I	*C. albicans*	ATCC 10259	1, 2, 3, 4, 5, 6, 7
	C. tropicalis	ATCC 7349	1, 2, 3, 4, 5, 6
	C. stellatoidea	ATCC 10264	1, 2, 3, 4, 5, 10, 32
V	*C. guilliermondii*	ATCC 7335	1, 2, 3, 4, 9
IV	*C. parakrusei*	ATCC 10232	1, 2, 3, 5, 13, 14, 15, c
III	*C. krusei*	NI 7492	1, 2, 5, 11, b
II	*C. pseudotropicalis*	NI 7494	1, 8, 10, 28, 31, a
Cryptococcus	*C. neoformans*	IFO 0410	Independent antigen

[a] ATCC, American Type Culture Collection, Rockville, Md.; NI, Nagao Institute, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan.

[b] The Arabic numeral indicates thermostable antigen and the letter, thermolabile antigen (26).

cine-HCl buffer (*p*H 8.0) containing 0.2 M NaCl, and the slurry was poured into a chromatographic column (2.5 × 40 cm) obtained from Pharmacia. All chromatographic experiments were carried out at 4 C, at a flow rate of 5 ml per hr adjusted by hydrostatic pressure. Amounts of 2 ml of about 2% γ-globulin samples were applied to the column.

In addition to gel filtration, diethylaminoethyl (DEAE)-Sephadex A-50 column chromatography was also used as a method for separation of the above γ-globulin, and for fractionation of labeled antibodies. After activating with 0.5 N NaOH, the gel was treated with the initial eluant, 0.02 M phosphate buffer (*p*H 6.6), until equilibrium was attained. The equilibrated ion-exchanger was packed into a chromatographic tube (1.5 × 25 cm), and approximately 5 ml of each sample, containing 0.5 to 1% protein, was applied to the column.

Analytical experiments. The electrophoretic mobility of each fraction was demonstrated by cellulose acetate electrophoresis by use of the method of Kohn (14). Veronal buffer (*p*H 8.6, 0.06 μ ionic strength), cellulose acetate (Millipore Corp., Bedford, Mass.) as the supporting medium, and staining with Ponceau 3R were used. Immunoelectrophoresis on glass slides was carried out by the micromethod of Scheidegger (17).

Fluorescent-antibody reaction. Purified γ-globulin fractions described in the text were adjusted to approximately 1% protein content and labeled with fluorescein isothiocyanate (FITC; BBL), as described by Riggs et al. (16), with dye-protein ratios of 1:50 and 1:25. Conjugation was carried out for 16 hr at 4 C. Conjugates were passed through Sephadex G-25 to separate free dye. Subsequent fractionation of conjugates were carried out on a DEAE-Sephadex column by stepwise elution with increasing concentrations of sodium chloride. Ordinary fluorescence microscope and filter accessories were used in these studies.

Preparation of crude extracted antigens. Three kinds of crude antigen fractions were used for immunizations. Crude polysaccharide was prepared according to the phenol-water method described by Westphal (29). Soluble polysaccharide and crude protein were prepared as follows. Yeast cells were grown for 48 hr on 2.5 liters of yeast carbon base medium (Difco) containing 0.5% ammonium sulfate at 27 C. The broth cultures were centrifuged, and supernatant fluids were concentrated 10 times in a rotary evaporator at 40 C. After dialysis against 0.067 M phosphate buffer (*p*H 7.4), the supernatant fluid was precipitated with one-fourth volume of 50% trichloroacetic acid. After centrifugation, the precipitate was washed twice with 10% trichloroacetic acid, washed with ether, and dried (crude protein fraction). The resulting supernatant fluid was dialyzed against tap water to remove the trichloroacetic acid and then dialyzed against distilled water. After dialysis, it was precipitated with 6 volumes of ethyl alcohol. The precipitate was washed with acetone and dried (soluble polysaccharide).

RESULTS

Fractionation of antiserum. Rabbit anti-*C. albicans* serum was precipitated with half-saturated ammonium sulfate. A 2-ml amount of the resulting γ-globulin solution, adjusted to 1% protein, was applied to a column containing about 200 ml of Sephadex G-200 equilibrated with 0.2 M Tris-0.5 M glycine-HCl buffer (*p*H 8.0) containing 0.2 M NaCl. Elution of γ-globulin solution with the same buffer yielded two peaks, PI and PII (Fig. 1). The antibody activity of the individual fractions was tested against homologous antigens by the tube agglutination method, and activity was found in the fractions composing peaks PI and PII.

A second fractionation of γ-globulin was carried out by DEAE-Sephadex column chromatography. A stepwise elution method was employed. The starting buffer (B1) was 0.02 M phosphate buffer (*p*H 6.6) containing 0.1 M NaCl; succeeding buffers contained 0.17 M NaCl (B2) and 0.3 M NaCl(B3). Three protein fractions (FI to FIII)

were obtained when buffers B1 to B3 were employed (Fig. 2). Antibodies were demonstrated in all the fractions, with strong reactivities in the FI and FIII.

The electrophoretic mobility of each of the fractions obtained by Sephadex G-200 column chromatography was examined by cellulose acetate electrophoresis. The PI fraction on a Sephadex G-200 column moved mainly to the β_1 and β_2 regions, and the PII protein moved to the γ_2 region. Similarly, the FIII fraction on DEAE-Sephadex moved to β_1 and β_2 regions, and the FI moved exclusively to the γ_2 region. Immunoelectrophoretic patterns of the fractions on Sephadex G-200 columns demonstrated that the PI fraction (collected as indicated in Fig. 1) contained IgM protein and a faster β component contaminating with a slight IgG, and that the PII fraction formed exclusively the IgG line. On DEAE-Sephadex columns, the FIII fraction contained IgM and a faster β component without association of IgG, and the FI fraction consisted exclusively of IgG. The FII protein obtained by use of a DEAE-Sephadex column moved to γ_2, β_2, and β_1 regions. Immunoelectrophoresis of the FII fraction indicated that the β_2 components were mostly IgA. This fraction was not tested for specificity in these studies.

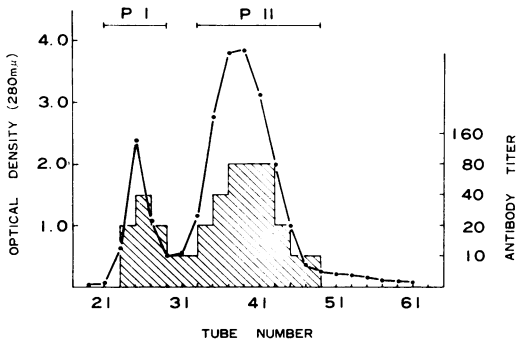

FIG. 1. *Gel filtration of γ-globulin of anti-Candida albicans serum by Sephadex G-200. The shaded area shows distribution of antibody activities measured by agglutination tests.*

Specificity of antibodies contained in the various fractions. Agglutinin activities of PI and PII fractions obtained by use of Sephadex G-200, as well as FIII and FI fractions obtained by use DEAE-Sephadex, from the γ-globulin of anti-*C. albicans* serum were titered against antigenically related species by the tube agglutination method (Fig. 3). Each fraction was generally collected in every experiment, as shown in Fig. 1 and 2, and each protein fraction was then concentrated to 0.1 to 1.0% protein content and diluted twofold. The antibody protein of the PI and FIII fractions reacted uniformly against the antigenically related species as well as the homologous species, demonstrating antibody titers ranging from 1:40 to 1:80 or from 1:160 to 1:320. However, the antibody protein of PII as well as FI reacted strongly to homologous species and to *C. tropicalis*, and weakly with the other five species, indicating that the predominant antibody activity was for antigen 7 or 6 of Tsuchiya's schema (26) and not for the other common antigens.

Fractionation of antiserum for fluorescent-antibody studies. The IgG of FI obtained by DEAE-Sephadex column chromatography from γ-globulin fraction was labeled with FITC at a

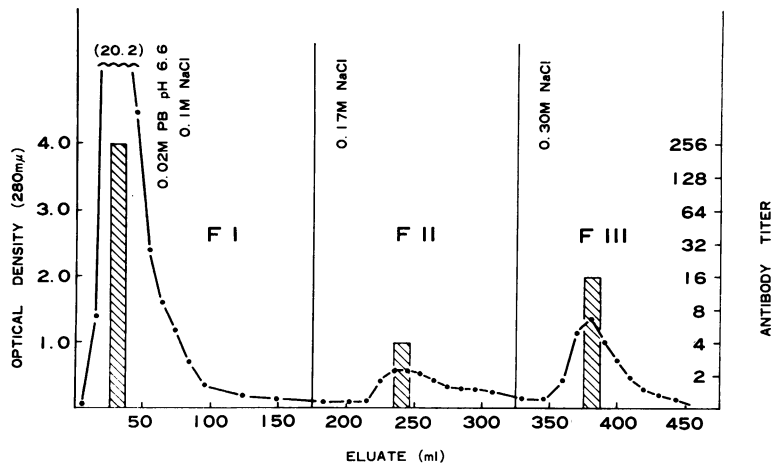

FIG. 2. *Separation of antibodies from γ-globulin fraction of anti-Candida albicans serum by DEAE-Sephadex. The shaded zones show agglutinin titers at each peak of protein fractions.*

dye-protein ratio of 1:50. The conjugate was fractionated again on DEAE-Sephadex by stepwise elution, as depicted in Fig. 4. The solid line indicates the absorption at 280 mμ, and the dotted line shows the absorption at 490 mμ. The FA reaction of the F3 and F4 fractions which were eluted with 0.02 M phosphate buffer containing 0.17 and 0.38 M NaCl, respectively, were remarkably clear and highly specific. The same fractionation procedure was carried out on the labeled IgM on a DEAE-Sephadex column, as depicted in Fig. 5. The FA staining of the conjugated F4 fraction, which was eluted with 0.02 M phosphate buffer containing 0.38 M NaCl, was of a weak intensity against cells of homologous and heterogeneous species.

Studies were performed on conjugated IgG to obtain a high yield of conjugate containing the species-specific factor. This reagent was subjected to adsorptions. The IgG purified by DEAE-Sephadex column chromatography from the γ-globulin fraction was labeled with FITC at a dye-protein ratio 1:25. This conjugate was then fractionated by DEAE-Sephadex column chromatog-

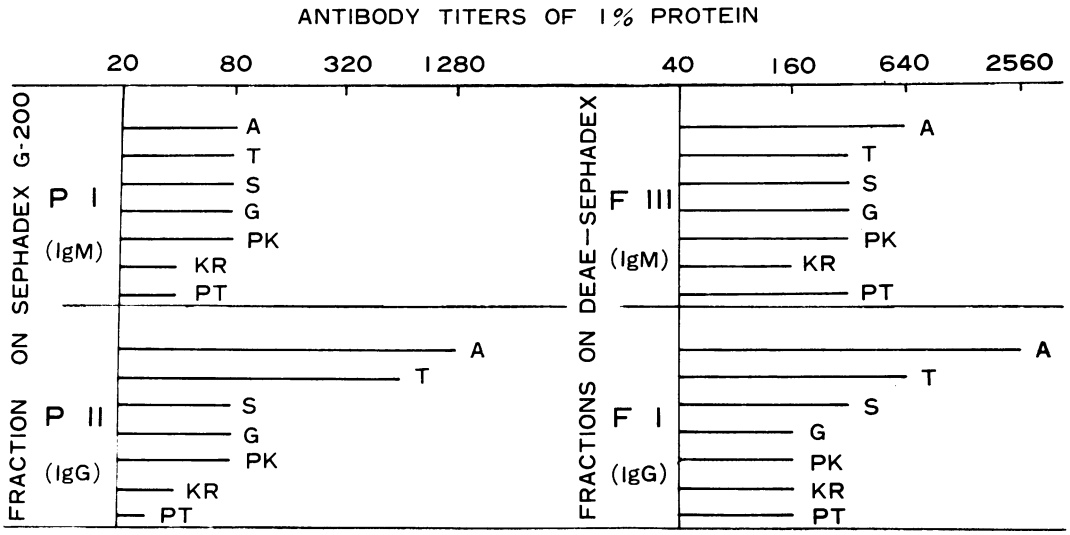

FIG. 3. *Comparison of antibody pattern of each fraction of anti-Candida albicans serum obtained by gel filtration and DEAE-chromatography. The IgM was responsible for antibody activities of PI and FIII fractions, and the IgG was responsible for PII and FI fractions. A, C. albicans; T, C. tropicalis; S, C. stellatoidea; G, C. guilliermondii; PK, C. parakrusei; KR, C. krusei; PT, C. pseudotropicalis.*

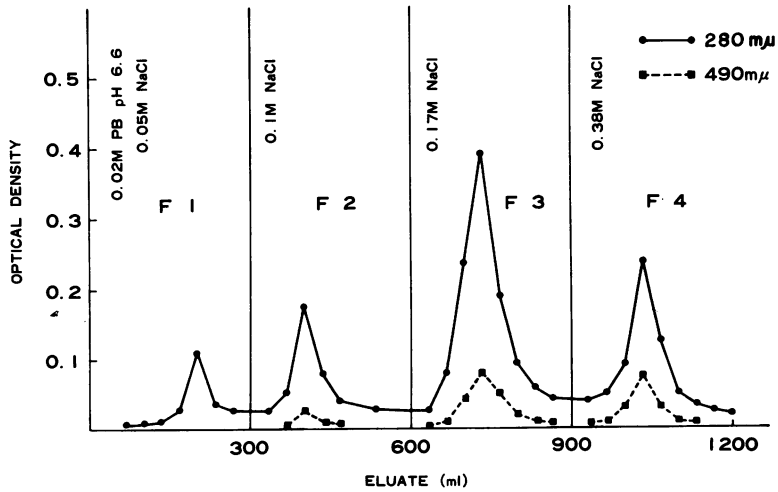

FIG. 4. *Fractionation of conjugated IgG of anti-Candida albicans serum by DEAE-Sephadex.*

raphy with the use of a stepwise elution method. The starting buffer (B1) was 0.02 M phosphate buffer (*p*H 7.5), and succeeding buffers contained 0.1 M NaCl (B2), 0.17 M NaCl (B3), 0.38 M NaCl (B4), and 1 M NaCl (B5). Four fractions (F2 to F5) were obtained (Fig. 6). A subfraction of F4 was an excellent reagent for the preparation of labeled factor antibodies, although this fraction was over-coupled. The yield and specificity of fluorescent antibodies prepared by the modifications of the above-mentioned methods are shown in Table 2. Only a few antigens were selected for determining the specificity of the reagents; these were *C. krusei*, *C. pseudotropicalis*, and *Crypto-*

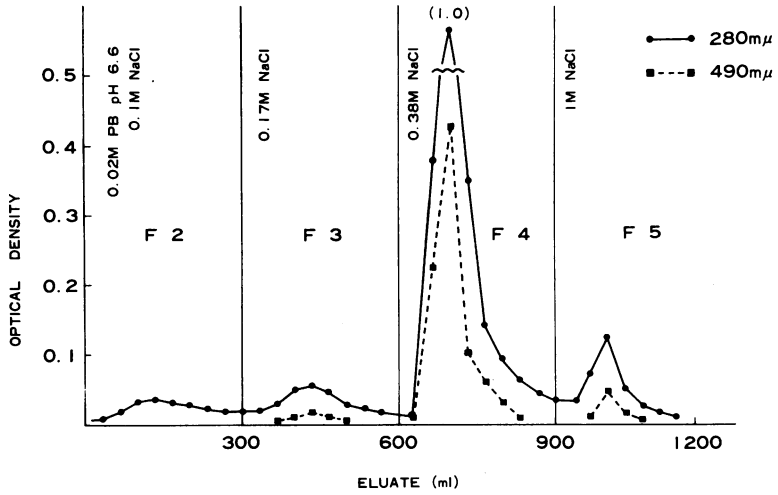

FIG. 5. *Fractionation of conjugated IgM of anti-Candida albicans serum by DEAE-Sephadex.*

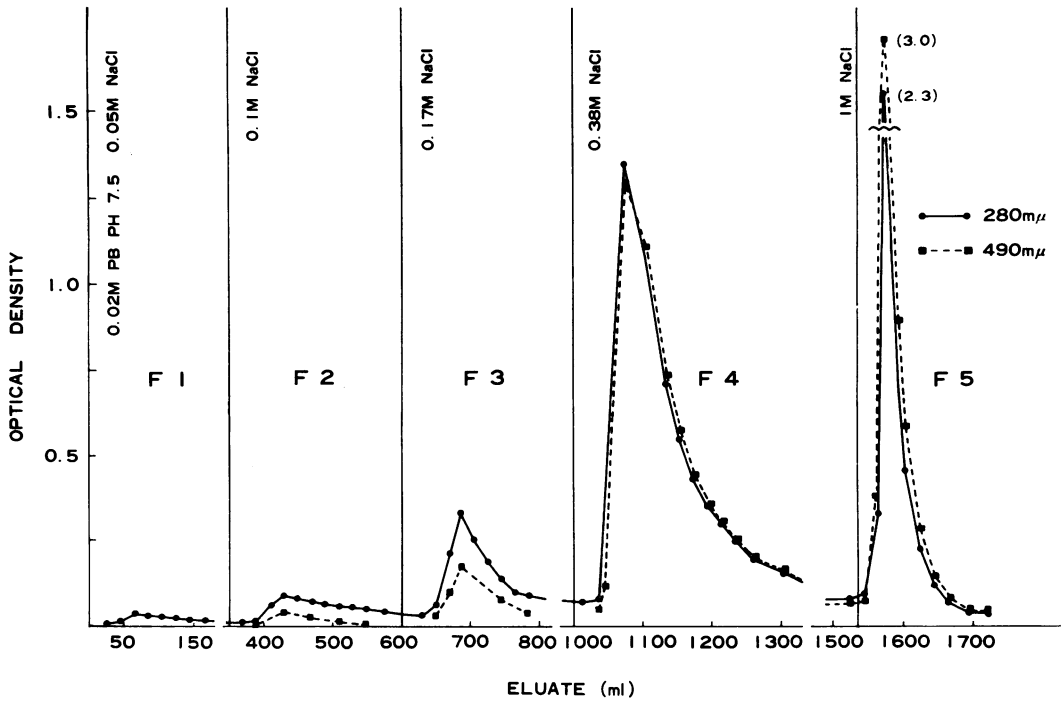

FIG. 6. *Fractionation of fluorescent IgG of anti-Candida albicans serum conjugated with dye at a high dye-protein ratio. The purified IgG was conjugated with FITC at a dye-protein ratio of 40 μg per mg of protein instead of 20 μg per mg of protein.*

coccus neoformans. The results indicated that the specificity of the labeled IgG was high in the fluorescent antibody as well as in the agglutination reaction, whereas that of labeled IgM was rather low. Furthermore, it was apparent that the labeled antibodies were not homogeneous with respect to the dye-protein ratio, and that several conjugated protein groups could be separated on a DEAE column, mainly according to the numbers of conjugated molecules of dye (Table 2). Over-coupled IgG conjugate with a high *p*H yielded a large amount of fluorescent antibody. Labeled factor antibody for antigen 6, specific to both *C. albicans* serotype C (tentatively designated) and *C. tropicalis*, could be prepared from labeled IgG (F1–F4) by adsorption with *C. stellatoidea*. Similarly, fluorescent factor antibody for antigen 7 of *C. albicans* type C was obtained from labeled IgG by adsorption with *C. tropicalis*, although this stained weakly. In contrast, fluorescent antibodies for antigens 6 and 7 were not present in the labeled IgM fraction, indicating the lesser specificity of the IgM (Table 3).

Responses and specificity of antibodies elicited by extracted antigens. Rabbits were immunized separately with each of the extracted antigens of crude polysaccharide, crude protein, and soluble polysaccharide suspended in Freund's complete adjuvant. Greater antibody formation was observed in rabbits immunized with crude poly-

TABLE 2. *Fractionation of fluorescent antibody and specificities of each fraction*

Column chromatography of labeled antibodies on DEAE-Sephadex		Concentrations of fluorescent antibodies measured by optical density at			Fluorescent-antibody reaction and slide agglutination against								Yields of fluorescent antibodies (ml) from 20 ml of antiserum
					Candida albicans		*C. krusei*		*C. pseudotropicalis*		*Cryptococcus neoformans*		
Fraction	NaCl (M)	280 mμ	490 mμ	F/P[a]	A[b]	F[c]	A	F	A	F	A	F	
FIII (IgM)													
F4	0.38	2.1	1.7	0.8	++	1	+	1	+	1	−	(1)[d]	3
F5	1.0	3.5	4.1	1.1	++	1	+	1	+	0	−	0	3
FI (IgG)													
F2	0.1	3.0	1.5	0.5	+++	3	+++	2	++	0	−	0	1
F3	0.17	6.1	4.0	0.6	+++	3	+++	2	++	1	−	0	2
F4	0.38	10.8	10.4	0.9	+++	3	+++	2	++	1	−	0	10
F5	1.0	3.9	4.4	1.1	+++	3	+++	1	++	0	−	0	5

[a] F/P: dye-protein ratio determined by relative value of optical densities at 490 mμ for FITC and at 280 mμ for protein.
[b] A: slide agglutination; +++ ~ + = degree of positive agglutination.
[c] F: Fluorescent-antibody reaction; 1~3 = degree of FA reaction.
[d] Weak reaction.

TABLE 3. *Specificities of fluorescent IgM and IgG of anti-Candida albicans serum*

Fluorescent-antibody fractions	Adsorption with	Antigen														Antibody factors concerned
		C. albicans		*C. tropicalis*		*C. stellatoidea*		*C. guilliermondii*		*C. parakrusei*		*C. pseudotropicalis*		*Cryptococcus neoformans*		
		A[a]	F[b]	A	F	A	F	A	F	A	F	A	F	A	F	
Fluorescent IgM (F4)	*C. stellatoidea*	±	1	±	1	−	0	−	0	−	0	−	0	−	0	(6[c])
	C. tropicalis	−	0	−	0	−	0	−	0	−	0	−	0	−	0	—
Fluorescent IgG (F3 or F4)	*C. stellatoidea*	+	2	+	2	−	0	−	0	−	0	−	0	−	0	6, 7
	C. tropicalis	+	1	−	0	−	0	−	0	−	0	−	0	−	0	7

[a] A: slide agglutination; +, ± = degree of positive agglutination.
[b] F: fluorescent-antibody reaction; 1, 2 = degree of fluorescent-antibody reaction.
[c] A small quantity of antibody to antigenic factor.

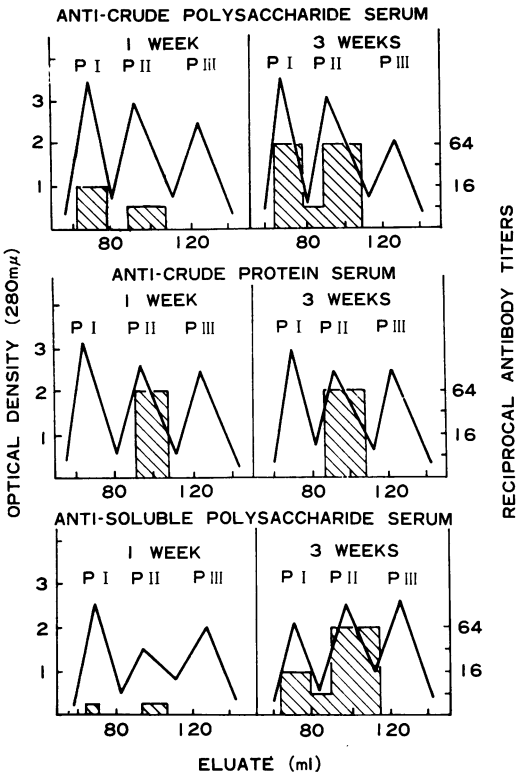

FIG. 7. *Responses of two types of antibodies to antigens of crude polysaccharide, crude protein, and soluble polysaccharide. The shaded squares show antibody activities tested by agglutination. The IgM and IgG are responsible for antibody activities of PI and PII fractions, respectively.*

saccharide than in rabbits immunized with crude protein. Responses of two types of immune globulins in antiserum after an injection of crude polysaccharide were observed to be relatively high in IgM and IgG at different time intervals, as shown in the upper part of Fig. 7. An injection of crude protein elicited a high response exclusively in the IgG, and an injection of soluble polysaccharide elicited a high response in IgG and a very low response in IgM. Antibody activities of the PI and PII fraction obtained from the anti-*C. albicans* crude polysaccharide serum were examined against antigenically related species by the tube agglutination method (Fig. 8). Antibody in the PI fraction reacted uniformly against the antigenically related species *C. tropicalis* and *C. stellatoidea*, as well as the homologous species. These patterns were significantly different from those of PII fraction, whereas the reactions to the heterogenous strains *C. parakrusei*, *C. krusei*, and *C. pseudotropicalis* were weaker than those of anti-whole cell antiserum. The antibody activity of the PII fraction demonstrated the heterogeneous reactivities to the antigenically related species, demonstrating high homologous specificity. Similarly, the PII fraction obtained either from the anti-crude protein serum or from the anti-soluble polysaccharide serum demonstrated the heterogenous reactions against antigenically related species. Their reactive patterns, however, were not exactly the same.

Adsorption experiments on the two types of antibodies obtained with various antigens. Specificities of the two types of antibodies were examined also by adsorption experiments. Each antiserum, without being salted out, was fractionated by gel filtration through Sephadex G-200. Elution of the whole serum yielded three protein fractions which showed three peaks, PI, PII, and PIII. Each antibody fraction was adjusted to yield an agglutination titer of 1:128 and was tested with the necessary antigens. An antibody pattern for antigen 6 was obtainable from PII fraction (IgG) from anti-whole cell serum, anti-crude polysaccharide serum, or anti-soluble polysaccharide serum, by adsorption with *C. stellatoidea*, but it was not

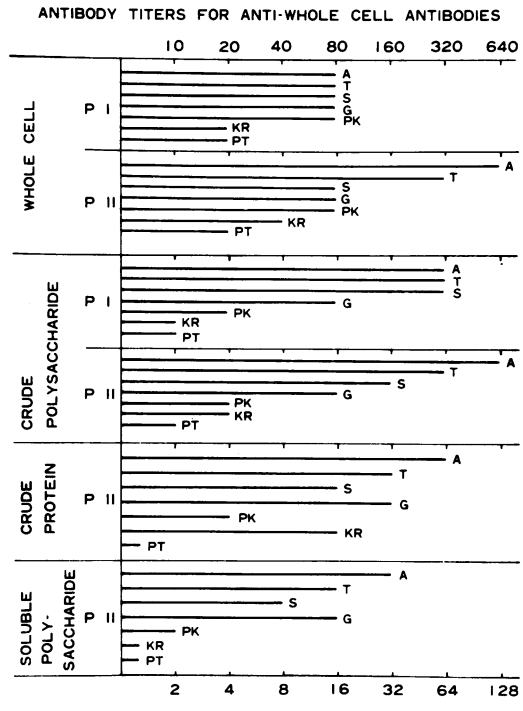

FIG. 8. *Reciprocal agglutination pattern of each antibody fraction produced by immunization with extracted antigens. A, Candida albicans; T, C. tropicalis; S, C. stellatoidea; G, C. guilliermondii; PK, C. parakrusei; KR, C. krusei; PT, C. pseudotropicalis.*

obtained from every PI fraction. Similarly, the antibody pattern for antigen 7 was obtainable from every PII fraction by adsorption with *C. tropicalis*, but not from every PI fraction (Table 4).

DISCUSSION

Serological classifications of yeasts based upon the work of several investigators are not in complete agreement. We attribute the discrepancies in results obtained for the same serological reactions to the varied nature of the antigens used, and to the variety of antibodies produced. Whole antiserum to date has generally been used for the serological detection of microorganisms. However, it has been shown that antibody proteins are not homogeneous, and that two types of antibodies, IgM and IgG, are important for serological reactions (1, 15). For investigation of the specificities of immune globulin fractions, the qualifications of the fractionation procedures must be known. In our comparative experiments with various methods, procedures in which ammonium sulfate-precipitated γ-globulin fraction and either gel filtration or DEAE-Sephadex column chromatography were used seemed to be reliable and practical enough for separation of the two types of immune globulins, although many chromatographic methods have been reported (7, 8, 19). These findings were similar to those of our previous experiments with anti-*Shigella flexneri* 1b and 2a sera. It has been shown that immunoelectrophoresis of the IgM fraction reveals the existence of a small IgA component (9), which occurred in several molecular classes (7). The importance of the IgA in agglutination is not completely clear. The IgA component contaminating the preparation of IgG or IgM plays little if any role in the specificity of the agglutination reaction. In the case of gel filtration, purity of the PI fraction depends on the range of fractions collected. Whole fraction of the PI, as indicated in Fig. 1, demonstrated the specificity of IgM in agglutination patterns and in adsorption experiments, although this whole fraction contained a slight IgG.

Different specificities were found in two types of immune globulins. The more specific reactions were manifested in IgG and less specificity was demonstrated in IgM by immunization with the different antigens studied. Moreover, specificities of all IgG antibodies produced by different antigen fractions, i.e., whole cell, crude polysaccharide, soluble polysaccharide, and crude protein were generally the same, although agglutination patterns of these antibodies were not exactly the same. As to the minor differences among the above IgG fractions, it is uncertain whether they would be caused by variations due to individual responses of the rabbits or to antigen fractions carrying slightly different determinant groups. Adsorption experiments proved that serotype-specific antibodies for *C. albicans* were obtainable from every IgG fraction but not from IgM. We consider that the specific sites of antigen for agglutination, even in crude protein antigen, should be a complex of specific polysaccharide terminal groups. Stewart-Tull et al. (21) reported that

TABLE 4. *Adsorption experiments with antisera immunized with extracted antigens*

Antigens for immunization	Antibody fractions	Adsorption with	Antigens for slide agglutination							Antibody factors concerned
			C. albicans	*C. tropicalis*	*C. stellatoidea*	*C. guilliermondii*	*C. parakrusei*	*C. krusei*	*C. pseudotropicalis*	
Crude polysaccharide	PI	*C. stellatoidea*	0	0	0	0	0	0	0	
	PII	*C. stellatoidea*	3[a]	3	0	0	0	0	0	6, 7
		C. stellatoidea + *C. tropicalis*	1	0	0	0	0	0	0	7
Crude protein	PII	*C. stellatoidea*	2	2	0	0	0	0	0	6, 7
		C. stellatoidea + *C. tropicalis*	1	0	0	0	0	0	0	7
Soluble polysaccharide	PII	*C. stellatoidea*	3	3	0	0	0	0	0	6, 7
		C. stellatoidea + *C. tropicalis*	1	0	0	0	0	0	0	7

[a] Numbers 1 $\sim$ 3 indicate degree of positive agglutination.

there were no differences in patterns for amino acid components in the strains of several genera of yeasts, and that the cell wall sugar components of these strains were different and demonstrated several patterns.

Several investigators (5, 11, 12) have studied the fluorescent-antibody reactions of *Candida* and other fungi, since Coons (3) introduced this technique. In the fluorescent-antibody reaction, attention has been paid to elimination of the nonspecific reactions of labeled antibody. We observed that elimination of IgM prior to conjugation with dye was very important for the purification of antibodies, because molecules of conjugate were not homogeneous with respect to dye-protein ratio, and subsequent fractionation of conjugate on DEAE ion-exchange columns were evidently dye-dependent (Table 2). It is assumed that labeled IgM as well as high molecular weight α or β components would play a role in nonspecific reactions, especially in tissue section examination. Labeled factor antibody for serotype diagnosis requires extensive adsorption for elimination of common antibodies, resulting in significant loss of specific antibodies. To resolve this difficulty, we used slightly over-coupled conjugates of IgG. Fractionation of the conjugate on a DEAE-Sephadex column with an eluant of relatively high *p*H, reflecting an increased isoelectric point (30), resulted in a high yield of fluorescent antibodies for preparation of factor-specific antibody after adsorption.

It was pointed out that time, route, dosage (28), and intensity (4) of immunization, and nature of antigens (Tsuchiya et al., Abstr. Intern. Congr. Microbiol., 9th, Moscow, 1966) are also important factors that influence the types of antibodies produced. Our results indicate that the response patterns of the two types of antibodies are different, depending upon the various antigen fractions injected. These patterns were indicative of the significant role of antigen molecules for eliciting antibody classes, and suggested that the use of soluble polysaccharide for immunization is suitable to obtain the IgG which possesses high homologous specificity. Immunizations of rabbits with different bacteria or yeasts demonstrated different patterns of antibody responses which were characteristic for each of the microorganisms studied. For example, the antibody response of rabbits to *Cryptococcus neoformans* was predominant in IgG (Tsuchiya et al., Abstr. Intern. Congr. Microbiol., 9th, Moscow, 1966), whereas O agglutinin for *Salmonella typhi* was found exclusively in the IgM fraction (15). These findings show the importance of realizing the immunogenic nature of antigenic molecules in the cell wall constituents when determining the responses of two types of antibodies.

We have observed some minor serological heterogeneity among species of *C. albicans*. However, we considered that typical strains of *C. albicans* were antigenically different, even if only slightly, from *C. tropicalis* and *C. stellatoidea*. Recently, a controversy arose on the serotype of *C. albicans* since Hasenclever reported the two serotypes of *C. albicans*, type A and type B. We tested the antigenic structure of *C. albicans* type B (supplied by J. Biguet) by slide agglutination test with our specific factor sera and found that *C. albicans* might be monotypic (26). Because this strain had a small amount of antigen 7 and a very small amount of antigen 6, it was considered to demonstrate a quantitative variation in specific antigens of *C. albicans*. More recently, upon testing several strains of *C. albicans* type A and type B from the National Communicable Disease Center, Atlanta, Ga., we considered that the strain without antigen 6, but with pathogenicity and chlamydospore-producing ability, ought to be regarded as important. However, we have not yet isolated such strains in our country. In the present study, we have used only type C strains, which prevail in Japan. Antigenic analyses of the three serotypes of *C. albicans* as well as related serotypes, *C. tropicalis* and *C. stellatoidea*, are in progress with the cooperation of L. Kaufman (National Communicable Disease Center). In any case, the demonstration of both antigens 6 and 7 is important for any typing of *C. albicans*.

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