ANTIGENIC STUDIES OF CANDIDA

II. Antigenic Relation of *Candida albicans* Group A and Group B to *Candida stellatoidea* and *Candida tropicalis*[1]

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ABSTRACT

HASENCLEVER, H. F. (U. S. Public Health Service, Bethesda, Md.), WILLIAM O. MITCHELL, AND JOSEPH LOEWE. Antigenic studies of Candida. II. Antigenic relation of *Candida albicans* group A and group B to *Candida stellatoidea* and *Candida tropicalis*. J. Bacteriol. **82**:574–577. 1961.—Previous work in our laboratory has shown the presence of two antigenic groups within the species *Candida albicans*. These groups have been designated as groups A and B. Subsequent studies have been conducted to show the antigenic relationship of these groups with *Candida stellatoidea* and *Candida tropicalis*.

Antisera to two strains each of *C. albicans* group A, *C. albicans* group B, *C. stellatoidea*, and *C. tropicalis* were used in this investigation. Individual suspensions from each of nine strains of *C. stellatoidea* and from ten strains each of the other groups and species were used to test the agglutinative properties of unadsorbed and adsorbed antisera. Separate samples of antisera to each immunizing strain were reciprocally cross adsorbed with the other immunizing strains and tested for agglutination.

The results indicated that *C. albicans* group A and *C. tropicalis* were antigenically identical and *C. albicans* group B and *C. stellatoidea* were antigenically indistinguishable.

A number of investigators have studied the antigenic relationships between *Candida albicans* and *Candida stellatoidea*. Martin (1942) and Jonsen, Thjotta, and Rasch (1953) found that a close antigenic similarity existed between these two species. Tsuchiya et al. (1954) and Rosenthal and Furnari (1958) observed antigenic differences between them. Gordon (1958), with fluorescent antibody staining procedures, was able to separate, on an antigenic basis, *C. albicans* and *C. stellatoidea*. Trimble (1957), using precipitation reactions, was able to show differences between these species. Most of these investigators agreed that *Candida tropicalis* had antigens in common with *C. albicans* and *C. stellatoidea*.

Hasenclever and Mitchell have reported (1960*a*) the observation of two antigenic groups in *C. albicans*. In this report we are presenting evidence showing the close antigenic similarity of *C. albicans* group A to *C. tropicalis*, and of *C. albicans* group B to *C. stellatoidea*.

MATERIALS AND METHODS

The strains of *C. albicans* used in this study were taken from a collection made for a previous study (Hasenclever and Mitchell, 1960*a*). *C. tropicalis* strains obtained from clinical specimens within the last year were used. Strains of *C. stellatoidea* were supplied by N. F. Conant, M. L. Gordon, M. Silva, and W. L. Wickerham. Since there had been some exchange of the strains of this species among these investigators, care was taken to avoid duplication. Three of the strains had been isolated within 2 years.

Antisera to two strains each of group A *C. albicans*, group B *C. albicans*, *C. stellatoidea*, and *C. tropicalis* were prepared as previously described (Hasenclever and Mitchell, 1960*b*). Reciprocal cross adsorptions of the antisera with each of the immunizing strains were done (Hasenclever and Mitchell, 1961). The results indicated, however, that adsorption of *C. stellatoidea* antisera with whole cell suspensions of *C. stellatoidea* or *C. albicans* group B did not remove all the agglutinating properties for *C. albicans*

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group A or *C. tropicalis*. This adsorption did remove the agglutinins for the homologous strain and for *C. albicans* group B strains. Therefore, washed fragments of yeast cells fractured by treatment with 20,000 to 25,000 lb/in^2 in a French press were used for adsorption. These fragments did remove all agglutinins for *C. albicans* group A or *C. tropicalis* cells.

Ten strains each of *C. albicans* group A, *C. albicans* group B, *C. tropicalis*, and nine strains of *C. stellatoidea* were used to test the agglutinating properties of each unadsorbed and adsorbed antiserum. The serological procedure was the same as used in an earlier report (Hasenclever and Mitchell, 1961).

RESULTS AND DISCUSSION

The results of agglutination of all strains studied with unadsorbed *C. albicans* group A and group B antisera are shown in Table 1. This illustrates the well-known concept of the common antigenicity that exists among these organisms.

Table 2 shows the results of agglutination with *C. stellatoidea* and *C. tropicalis* antisera and again indicates the antigenic similarity of these organisms.

The agglutination reactions of nine strains of *C. stellatoidea* by adsorbed antisera are shown in Table 3. It should be emphasized that the results presented in this table represent the data obtained from studies with antisera to two strains of each group or species, and that there was

TABLE 1. *Agglutination reactions* with unadsorbed Candida albicans group A and group B antisera*

Candida species	Antisera to			
	C. albicans group A		*C. albicans* group B	
	Strain 207	Strain B311	Strain 526	Strain 792
C. albicans group A	480–960	480–960	240	240–480
C. albicans group B	480–960	480	240–480	480–960
C. stellatoidea	480–960	240–480	240–480	480
C. tropicalis	480–960	480–960	240–480	240–480

* The reciprocal of the serum dilution.

The values in the table express the range of agglutination of each antisera for all the strains of each species or group studied.

TABLE 2. *Agglutination reactions* with unadsorbed Candida stellatoidea and Candida tropicalis antisera*

Candida species	Antisera to			
	C. stellatoidea		*C. tropicalis*	
	Strain 2	Strain 3	Strain 48	Strain 3166
C. albicans group A	960	960	480–960	960
C. albicans group B	960	960	240	480–960
C. stellatoidea	960	960	240	240–480
C. tropicalis	960	960	960–1920	960

* The reciprocal of the dilution.

The values in the table express the range of agglutination of each antiserum for all the strains of each species or group studied.

TABLE 3. *Agglutination reactions* of 9 strains of Candida stellatoidea with adsorbed Candida species antisera*

Antiserum to	Adsorbed with			
	C. albicans group A	*C. albicans* group B	*C. tropicalis*	*C. stellatoidea*
C. albicans group A	Neg	Neg	Neg	Neg
C. albicans group B	Neg	Neg	Neg	Neg
C. stellatoidea	Neg	Neg	Neg	Neg
C. tropicalis	Neg	Neg	Neg	Neg

* No agglutination at a serum dilution of 1:30.

reciprocal adsorption of each antiserum with each immunizing strain employed. Therefore, as an example, antisera to *C. albicans* strains 207 and B311 (group A) were adsorbed individually with yeast-cell suspensions of strains 207 and B311. The nine *C. stellatoidea* strains were then tested for agglutination by these four adsorbed antisera. The results of the other combinations that appear in this table were obtained in the same manner. It appears, by the methods used here, that *C. stellatoidea* does not possess any specific antigens, at least when compared to these other two species.

The agglutinating properties of these adsorbed antisera for ten strains of *C. tropicalis* are presented in Table 4. *C. albicans* group A antisera adsorbed with *C. albicans* group B or *C. stellatoidea* cells agglutinated *C. tropicalis* cells. *C. tropicalis* antisera adsorbed with *C. albicans*

TABLE 4. *Agglutination reactions* of 10 strains of Candida tropicalis with adsorbed Candida species antisera*

Antiserum to	Adsorbed with			
	C. albicans group A	*C. albicans* group B	*C. tropicalis*	*C. stellatoidea*
C. albicans group A..	Neg	60–120	Neg	60–120
C. albicans group B..	Neg	Neg	Neg	Neg
C. stellatoidea.....	Neg	Neg†	Neg	Neg†
C. tropicalis.....	Neg	120–240	Neg	120–240

* Reciprocal of serum dilution, or negative at serum dilution 1:30.

† Required eight adsorptions with broken cell fragments to remove agglutination for *C. tropicalis* cells.

group B or *C. stellatoidea* cells agglutinated *C. tropicalis* antigenic suspensions. Adsorption of group B or *C. stellatoidea* antisera with suspensions of any of the groups or species included in this study removes their agglutinating properties. The agglutination of *C. tropicalis* antisera for *C. tropicalis* strains could be removed by homologous adsorption or with *C. albicans* group A cells.

Table 5 shows the reactions of adsorbed antisera for ten *C. albicans* group A strains. The results obtained for these strains are almost identical with those shown in Table 4 for *C. tropicalis* strains and indicate a close antigenic relationship.

The agglutination of *C. albicans* group B strains is presented in Table 6. These data, identical with that shown in Table 3, suggest a close antigenic resemblance between group B and *C. stellatoidea* strains.

It is quite apparent from the results shown here that the agglutinating properties of *C. albicans* group B and of *C. stellatoidea* antisera can be removed by adsorption with yeast cells of any of the species or groups studied. Adsorption of *C. albicans* group A or *C. tropicalis* antisera with either *C. albicans* group A or *C. tropicalis* cells removes their agglutinating activity for all the groups and species. However, *C. albicans* A or *C. tropicalis* antisera still showed agglutinating activity for either group A or *C. tropicalis* yeast cells after adsorption with either group B or *C. stellatoidea* cells. *C. albicans* group B or *C. stellatoidea* cells were not agglutinated by this adsorbed antisera.

The antigenic relationship of *C. albicans* and *C. tropicalis* has been known for many years. Benham (1931) showed that *Monilia albicans* and *Monila candida* (synonym of *C. tropicalis*) possessed common antigens. Almon and Stovall (1934) in a more comprehensive study observed the same results and suggested that *M. candida* was a diphasic form of *M. albicans*. These species are now, of course, recognized as being separate, but the study illustrates the early recognition of the antigenic similarity between some strains of *C. albicans* and *C. tropicalis*.

It is difficult to explain the inability of unbroken suspensions of *C. stellatoidea* to remove by adsorption all the agglutinating properties for *C. albicans* group A or *C. tropicalis* cells of *C. stellatoidea* antisera. Whole *C. albicans* group B cells were also ineffective. Agglutination for

TABLE 5. *Agglutination reactions* of 10 strains of Candida ablicans group A with adsorbed Candida species antisera*

Antiserum to	Adsorbed with			
	C. albicans group A	*C. albicans* group B	*C. tropicalis*	*C. stellatoidea*
C. albicans group A...	Neg	60–120	Neg	60–120
C. albicans group B...	Neg	Neg	Neg	Neg
C. stellatoidea.....	Neg	Neg†	Neg	Neg†
C. tropicalis.....	Neg	60–120	Neg	60–120

* Reciprocal of serum dilution or negative at serum dilution 1:30.

† Required eight adsorptions with broken cell fragments to remove agglutination for group A cells.

TABLE 6. *Agglutination reactions* of 10 strains of Candida albicans group B with adsorbed Candida species antisera*

Antisera to	Adsorbed with			
	C. albicans group A	*C. albicans* group B	*C. tropicalis*	*C. stellatoidea*
C. albicans group A...	Neg	Neg	Neg	Neg
C. albicans group B...	Neg	Neg	Neg	Neg
C. stellatoidea.....	Neg	Neg	Neg	Neg
C. tropicalis.....	Neg	Neg	Neg	Neg

* No agglutination at serum dilution 1:30.

group B or *C. stellatoidea* cells with *C. stellatoidea* antisera was removed by adsorption with whole cells. It seems most likely that agglutinins to antigens residing deep and not on the surface of *C. stellatoidea* cells were present in the antisera and that adsorption with whole cells did not remove these antibodies. *C. albicans* group A and *C. tropicalis* possess these antigens on their cell surfaces and therefore agglutinate. The fact that broken group B or *C. stellatoidea* cells removed these agglutinating properties indicates the plausibility of this hypothesis. The antigen(s) was available in the fractured cells, for combination, and the antibody was removed by the adsorption procedure. However, this phenomenon was not observed with antisera to *C. albicans* group B.

Controls were also carried out adsorbing *C. albicans* group A and *C. tropicalis* antisera with cell fragments of group B or *C. stellatoidea* but the results were the same as adsorption of these antisera with intact cells. Antisera obtained from rabbits following immunization with broken cells gave the same results as antisera to whole cell suspensions.

Other methods, as described previously (Hasenclever and Mitchell, 1961), have been tried to show the antigenic differences indicated in this study, but have proved unsuccessful.

It seems quite certain that one of the main reasons that these antigenic groups have not been observed before is because of the method employed. We have not been able to demonstrate it except with tube agglutination. Slide agglutination is less reliable in showing these differences than tube agglutination. Since the antigenic groups exist in roughly equal numbers, it is very possible that investigators would inadvertently choose only a group A or a group B strain for the production of antiserum. In a previous study, Hasenclever and Mitchell (1960*a*) used two strains of *C. albicans* for producing antisera and fortuitously chose two group A strains. Had we chosen two group B strains or one of each group for immunization, the results and conclusions would have been much different.

The serum dilution schedule used in this work also deserves some comment. It is quite possible, since we used a final dilution of 1:30 in the first tube, that subtle antigenic differences were missed. However, this method emphasizes the more obvious differences. There is little question that *C. albicans* group A and *C. tropicalis* are very similar antigenically. Likewise, *C. albicans* group B and *C. stellatoidea* appear to be antigenically homogeneous, but *C. albicans* group A and *C. tropicalis* apparently possess equal but additional antigenic components with respect to the other group or species.

This investigation clarifies some of the confusion with regard to the antigenic relationships of these organisms.

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