ANTIGENIC STUDIES OF CANDIDA

I. OBSERVATION OF TWO ANTIGENIC GROUPS IN Candida albicans

H. F. HASENCLEVER and WILLIAM O. MITCHELL
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service, Bethesda, Maryland

Received for publication April 17, 1961

ABSTRACT

HASENCLEVER, H. F. (U. S. Public Health Service, Bethesda, Md.), AND WILLIAM O. MITCHELL. Antigenic studies of Candida. I. Observations of two antigenic groups in Candida albicans. J. Bacteriol. 82:570-573. 1961.—Two distinct antigenic groups, detected by tube agglutination, have been observed among strains of Candida albicans. Adsorption of an antiserum with a heterologous strain did not remove the agglutinating properties for the homologous strain. Homologous adsorption of the antiserum did remove its agglutinating properties. Seventy-one isolates of C. albicans have been screened with this adsorbed antiserum and 38 were agglutinated at a serum dilution of 1:240 to 1:480 (group A), whereas 33 were not agglutinated at a serum dilution of 1:30 (group B). Thirty-five of the strains were studied with rabbit antiserum prepared against each of six strains (three from each group). Agglutination reactions of these strains with samples of each antiserum adsorbed individually with each immunizing strain verified the results of the screening agglutination reactions. All the agglutinating properties of antiserum prepared against the three group B strains were removed by adsorption with suspensions of either group A or group B strains. The identity of all isolates used in this study was confirmed by chlamydoospore formation, fermentation, and carbohydrate assimilation reactions.

In this report we show evidence indicating the presence of two antigenic groups in Candida albicans. The results, upon which these conclusions are based, were obtained from tube agglutination reactions.

This study was prompted by the observation that the agglutinating properties of an antiserum to one strain of C. albicans, when adsorbed with the cells of a different isolate of the same species, were not removed for the homologous strain. An investigation of more isolates indicated that some were agglutinated by this adsorbed antiserum whereas others were not. The strains that were, agglutinated have been designated as group A, whereas those that were not agglutinated are group B.

MATERIALS AND METHODS

All the strains of yeasts included in this study conform to the morphological and physiological characteristics of C. albicans. Seventy-one strains (62 recent isolates and 9 laboratory strains) have been screened with an adsorbed antiserum to determine their antigenic group. Thirty-five of these strains have been studied extensively with unadsorbed and adsorbed antisera.

Antisera to six strains of C. albicans (three group A strains and three group B strains) were produced in rabbits following the intravenous injection of either viable or heat-killed suspensions of yeast cells. The schedule of immunization was the same as described previously (Hasenclever and Mitchell, 1960).

Each antiserum was adsorbed separately with each of the six strains employed in the production of antisera. Equal volumes of antiserum and 50% heat-killed yeast suspensions were mixed and placed in a water bath for 3 to 5 hr at 45 C. The mixture was centrifuged and the serum was removed. This process was repeated three more times. The first and third adsorptions were done at 45 C, whereas the second and fourth adsorp-

1 Presented at the 1960 Annual Meeting of the Society of American Bacteriologists.
tions were carried out overnight at refrigerat
temperatures. Following completion of the fourth
adsorption, the serum was removed, diluted 1:3,
and used for serological studies.

Antigens utilized for agglutination reactions
were dilute suspensions of heat-killed yeast cells.
Suspensions used for agglutination contained
approximately $3 \times 10^6$ yeast cells per ml.

Standard agglutination procedures were used,
and the lowest serum dilution was 1:30. The
serum antigen mixtures were placed in a water
bath at 45 C for 2 to 3 hr, refrigerated overnight,
and read the following morning.

RESULTS AND DISCUSSION

Screening of the 71 strains was done with
group A antiserum that had been adsorbed with
a group B strain. Those results are shown in
Table 1. Thirty-eight strains (group A) were
agglutinated at a serum dilution of 1:240 to 1:480,
whereas 33 strains (group B) were not aggluti-
nated at 1:30.

Nineteen group A strains and 16 group B
strains were studied extensively with unadsorbed
and adsorbed antisera. Agglutination reactions
of these strains with unadsorbed antisera may
be seen in Table 2. The top line of the table in-
dicates with each antiserum the range of agglu-
tination titers for all 35 strains. The bottom line
shows the number of strains agglutinated at each
serum dilution and their antigenic category. An-
tiser to group A strains B311 and 857 show
little difference in their agglutinating properties
for either group A or group B strains, whereas
antisum 207 (group A) agglutinates, at a
higher titer, most of the group A strains. Anti-
sera to group B strains agglutinate, at a higher
titer, more of the group B strains than the group
A strains.

Table 3 shows the agglutination reactions of
the six antisera following adsorption with group
B strains. The results indicate that this adsorp-
tion of group A antisera reduces the titer for
group A strains; however, the titer is the same
regardless of which group B strain was used. The
agglutinating properties of group A antisera for
group B strains were removed by this adsorption.
Adsorption of group B antisera with group B
strains removed its agglutination for either
group.

The results showing the effect of adsorption
with group A strains of group A and group B
antisera are presented in Table 4. This adsorp-
tion of the antisera removes, for either group,
all their agglutinating properties.

A summary of agglutination reactions with
adsorbed antisera is shown in Table 5. The figures
indicated by the asterisk represent the observed
upper and lower extremes. The majority of these
reactions were titers of 1:120 to 1:240.

The antigenic differences described in this
report have been observed utilizing tube agglu-
tination as the serological procedure. Studies
with other techniques for demonstrating antigen-

\[\text{TABLE 1. Agglutination of 71 strains by screening antiserum}\]

<table>
<thead>
<tr>
<th>Group</th>
<th>Laboratory strains</th>
<th>New isolates</th>
<th>Total strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A*</td>
<td>6</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Group B†</td>
<td>3</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>62</td>
<td>71</td>
</tr>
</tbody>
</table>

* Agglutination at 1:240 or 1:480 serum dilutions.
† No agglutination at 1:30 serum dilution.

\[\text{TABLE 2. Agglutination reactions of 35 strains* with unadsorbed antisera}\]

<table>
<thead>
<tr>
<th>Strain (antigenic group) at indicated titers† of antisera:</th>
</tr>
</thead>
<tbody>
<tr>
<td>B311(A) 207(A) 857(A) 792(B) 3171(B) 526(B)</td>
</tr>
<tr>
<td>480 960 240 480 960 480 960 1920 120 240 480 120 240 480 120</td>
</tr>
</tbody>
</table>

| No. strains of group A | 17 | 2 | 1 | 1 | 17 | 16 | 3 | 16 | 2 | 9 | 10 | 7 | 12 |
| No. strains of group B | 16 | 14 | 2 | 3 | 13 | 3 | 13 | 2 | 7 | 7 | 1 | 4 | 11 |

* Group A strains, 19; group B strains, 16.
† Reciprocals of serum dilutions.
made, but have not been consistent in showing the differences observed with agglutination reactions. Culture filtrates, French press extracts, extracts of candida cells ground with alumina, and acid hydrolyzates have been investigated for antigenic activity, but without reproducibility.

There has been some question as to the validity of agglutination reactions with yeast cells. Martin (1942) concluded that agglutination with Candida species was unreliable, and preferred to use precipitation or complement fixation. Other investigators, Tsuchiya et al. (1954), Jonsen, Thjotta, and Rasch (1953), and Rosenthal and Furnari (1958), have obtained consistent and reproducible results with agglutination. The latter has been our experience with this, and other studies (Hasenclever and Mitchell, 1960).

A serum dilution of 1:30 as the lowest dilution was used to obviate possible nonspecific reactions. Heating serum at 56 C for 30 min also helped eliminate these factors. Heated, pooled normal rabbit serum diluted 1:5 did not agglutinate any of 20 strains tested.

Table 3. Agglutination reactions of 35 strains* with antisera adsorbed with group B strains

<table>
<thead>
<tr>
<th>Group</th>
<th>Antiserum</th>
<th>Adsorbed with</th>
<th>Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Group B</td>
<td>Group B cells</td>
<td>60-480*</td>
</tr>
<tr>
<td>B</td>
<td>Group A</td>
<td>Group A cells</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>Group B cells</td>
<td>&lt;30</td>
</tr>
<tr>
<td></td>
<td>Group B</td>
<td>Group A cells</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

* These figures represent the extreme range of positive titers as shown in Table 3. The majority of the reactions were from 120 to 240.

Seventy-one strains of C. albicans have been categorized as to group. Of these isolates all have been found to fall within group A or B. Antisera to six strains, three group A and three group B, unadsorbed and reciprocally adsorbed, were utilized. Thirty-five strains, 19 group A and 16 group B, were employed for agglutination reactions with the unadsorbed and cross-adsorbed antisera. There were quantitative differences existing among the antisera, both before and after adsorption, but the reactions were consistent and reproducible.

It is apparent that, under the experimental conditions described, the group A strains possess an antigen or antigens that are not present in the group B strains, but still contain all of those associated with group B isolates. It is the presence of this antigen or antigens in the group A strains and the absence of the antigen(s) in group B strains that make these two groups distinguishable. Strains of C. albicans can be easily separated into their respective groups using tube agglutination reactions and adsorbed antisera.

LITERATURE CITED


