Cross-Agglutination Reaction in Candida albicans and Enteric Bacilli

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Cross-reactivity between Candida albicans and representative Enterobacteriaceae was investigated by agglutination methods. It was observed that anti-Candida serum reacted to certain groups of salmonellae and shigellae. The only antiserum against the enteric bacilli that reacted with Candida was Salmonella C. When anti-Candida serum was adsorbed with C. albicans or S. montevideo, all the activity was removed. However, when the anti-Salmonella serum was adsorbed with the antigens, Salmonella adsorbed the whole antibody activity, whereas Candida removed only its corresponding antibody.

The occurrence of agglutinins to Candida albicans in human sera has been reported previously by a number of investigators (2, 4, 8, 9, 11). Controversial opinions exist as to the source of this antibody. Winner (13) has stated that these agglutinins are the result of clinical or subclinical infections with C. albicans or antigenically related organisms. According to Drake (4), however, these agglutinins are "normal" serum constituents whose ability to react with C. albicans is fortuitous. It is unlikely that the high titer of anti-Candida agglutinins in the sera of normal individuals is due to subclinical infections with Candida, as Maibach and Kligman (6) showed that the titer of agglutinins against C. albicans was not elevated in experimentally superficially infected individuals.

Cross-reactivity between Candida and Saccharomyces has been reported before (9), but similar evidence is not available in regard to Candida and bacteria. In this study, attempts were made to investigate cross-reaction in C. albicans and several enteric bacilli.

MATERIALS AND METHODS

Microorganisms. The bacteria used in this study have been isolated from patients and were identified biochemically and serologically. The strain of Salmonella C used for immunization of rabbits as well as for agglutination tests was isolated from a patient suffering from salmonelllosis. It was typed in our laboratory and further identified by the National Communicable Disease Center (NCDC) as Salmonella montevideo. The strain of C. albicans used in this study was received from the NCDC. It was kept in our laboratory by subculturing on Sabouraud glucose-agar for over 1 year.

Immunization procedures. Antigens for immunization were prepared from S. montevideo grown on nutrient agar and C. albicans grown on Sabouraud dextrose-agar for 24 hr. The surface growth was suspended in saline and washed twice with sterile physiological saline. The suspension was heated at 63 C for 0.5 hr, and was adjusted to contain approximately 3 X 10^10 Candida cells or 32 X 10^9 Salmonella cells. Two rabbits were used for preparation of antiserum against Salmonella. Each was injected with 0.5 ml of the antigen suspension intravenously and 0.5 ml of antigen subcutaneously at the same time. The injections were repeated five times at 7-day intervals. For preparation of C. albicans antiserum, a rabbit was injected with 0.5 ml of the antigen intravenously and 0.5 ml subcutaneously. Injections were repeated five times at 3- to 4-day intervals.

Agglutination procedure. Agglutination was performed by both slide and tube methods using live microorganisms as antigens. The turbidity of the antigens used for agglutination was approximately the same as the turbidity of the immunogens. All of the antisera used for screening slide tests were obtained from Difco, with the exception of anti-Candida serum, which was prepared in our own laboratory.

Adsorption technique. The anti-Candida and anti-Salmonella sera were diluted 1:20 with saline, and were adsorbed with the live homologous and the heterologous organisms. The amount of antigen used for adsorption was approximately 40 mg (wet weight) per ml of diluted antiserum. Adsorption was performed for 2 hr at 37 C, followed by incubation at 4 C overnight.

RESULTS

Screening test. Table 1 presents the results of the slide test in which various antisera against several enteric bacilli and Candida were examined with the homologous and heterologous organisms. The results show that anti-Candida sera gave a slide agglutination reaction not only with
the corresponding antigen but also with Salmonella A, B, C₁, and D, as well as with Shigella B and D. No agglutination was observed with other microorganisms tested. However, when antisera from different groups of Salmonella and Shigella were tested with various enteric bacilli as well as with Candida, the results were different. The only antiserum which gave agglutination with C. albicans was anti-Salmonella C₁ serum.

Titration. In slide agglutination tests which gave positive reactions, tube methods were employed for titration of antibody by using antisera which were prepared in our laboratory. Table 2 presents the results of titration of anti-Salmonella and anti-Candida sera against homologous and heterologous species. It shows that anti-Salmonella serum has different titers with various organisms tested, whereas anti-Salmonella C₁ serum has the same titer with C. albicans as with the corresponding antigen.

The titer of anti-Salmonella serum against the heterologous or homologous organisms was considerably higher than the titer of anti-Candida serum. This difference may be due to the number of organisms used to obtain the antiserum, the difference in the immunization schedule, or a difference in the antigenicity of the microorganisms.

Adsorption. Both anti-Candida and anti-Salmonella sera were adsorbed with the corresponding as well as heterologous antigens. As shown in Table 3 in the case of anti-Candida sera, the antibody activity against Candida and Salmonella was removed when adsorbed with either antigen. However, when anti-Salmonella serum was adsorbed with the same antigens, C. albicans removed only its corresponding antibody, whereas Salmonella C₁ removed antibody activity against both organisms.

### DISCUSSION

From the earliest days of immunology, it has been observed that normal sera frequently contain agglutinins, lysins, and complement-fixing antibodies for bacteria, red cells, and fungi (1, 5, 10). Up to the present time, no completely acceptable explanation has been proposed to account for the existence of such antibodies. Landsteiner (5) mentioned two hypotheses to account for the occurrence of these antibodies. (i) These antibodies in general are of spontaneous origin. (ii) These antibodies are present as a consequence of previous antigenic stimulation with the test antigen or with foreign macromolecules that share determinant groups with the test antigen.

With regard to the former theory, if these antibodies are of spontaneous origin, it would be difficult, though not impossible, to account for their specificity and for the variations from one individual to another. In support of the view that these antibodies are of immune origin, there is much evidence that many antigenic determinant groups, particularly those occurring on polysaccharides, are shared among many microorganisms.
as well as animal and plant tissues (12). Neil et al. (7) reported that a serological cross-reaction exists between *Sporotrichum schenckii* and *Diplococcus pneumoniae*, and this cross-reactivity resides on the capsular polysaccharide of pneumococci.

This report lends support to the second theory, that is, the antibody against the microorganism arises as a result of previous immunization. On the other hand, it is clear that antigenicity of *Candida* is low, and, as Maibach and Kligman (6) reported, upon repeated infections in human subjects with *C. albicans*, the titer of antibody did not increase. Moreover, it is assumed that immunological stimulus caused by many superficial infections is inadequate to induce a significant titer of antibody. The present study shows that the reciprocal cross-agglutination reaction occurs between *C. albicans* and *S. montevideo*. Reciprocal reactions did not occur, however, between *Candida* and other salmonellae and shigellae. Although anti-*Candida* serum gave a positive agglutination reaction with *Salmonella* A, B, and D and *Shigella* B.D., the antibody against these microorganisms did not react with *C. albicans*. The chemical structure of O determinant of *Salmonella* and the wall of *Candida* are relatively similar (3). This might account for this cross-reactivity.

When, anti-*Candida* serum was adsorbed with the homologous or cross-reacting organism, the whole antibody activity was removed. However, when attempts were made to adsorb anti-*Salmonella* serum with *Candida*, antibody activity against the heterologous organism was decreased but not completely removed. This partial removal of anti-*Salmonella* serum with *Candida* suggests that *Salmonella* antigen contains, in addition to the common determinants, some other antigenic determinants which induce an antibody specific for *Salmonella* and, therefore, this antibody was not removed upon adsorption with the heterologous antigen.

**LITERATURE CITED**