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Candida albicans Group A-Specific Soluble Antigens Demonstrated by Quantitative Immunoelectrophoresis

ROLAND M. F. GUINET* AND SYLVIE M. GABRIEL

Service de Mycologie, Institut Pasteur de Lyon, 69365 Lyon Cédex 2, France

Soluble cytoplasmic extracts of *Candida albicans* groups A and B were prepared and compared by quantitative immunoelectrophoresis experiments performed with a commercial anti-*C. albicans* group A immune serum. Although crossed immunoelectrophoresis, tandem crossed immunoelectrophoresis, and line immunoelectrophoresis revealed many cross-reactions between the two groups, some components seemed to be specific to group A. However, the complexity of the extracts studied did not allow us to demonstrate specific constituents with these methods. Crossed-line immunoelectrophoresis with and without absorption of antibodies in situ was then used, and four specific antigens unique to group A cytoplasmic extract were demonstrated, one of which appeared to be quantitatively important. The value of various quantitative immunoelectrophoretic methods applied to complex antigenic preparations is discussed.

In 1961, Hasenclever and Mitchell (11) showed that *Candida albicans* strains could be divided by agglutination-absorption experiments into two types, of which type A contained at least one antigen more than type B on yeast-phase cells. Subsequent studies have been conducted to show the antigenic relationship of *C. albicans* to *Candida stellatoidea* and *Candida tropicalis*. The results (12) indicated a close antigenic similarity of *C. albicans* group A to *C. tropicalis* and of *C. albicans* group B to *C. stellatoidea*. Tsuchiya et al., using cross-absorption, identified several antigenic factors and determined the existence of a "C" serotype for *C. albicans* (24). However, difficulties are frequently encountered in serotyping *C. albicans* strains, and the existence of intermediate strains was demonstrated by Vernes et al. (25). Furthermore, recently Taguchi et al. (22), using monospecific agglutination antisera, did not observe agglutination in 45 strains of *C. albicans* serotype A with the 13b antigen antiserum. Thus, they could not differentiate *C. albicans* and *C. tropicalis* on the basis of their agglutinating antigens, and for antigenic relationship studies agglutination-absorption experiments had limited possibilities.

The above results were obtained with whole cells, so the information related only to the surface antigens. Complex antigen extracts containing the water-soluble cytoplasmic antigens have been prepared by disruption of cells and removal of insoluble material. Such preparations are the best available reagents for gel precipitation tests for diagnosis of systemic candidiasis (4). The number of antigens detected in such extracts ranges from 5 to 7 by double diffusion in gel (6, 23) through 15 to 17 by classical immunoelectrophoresis (7, 8) to 68 to 78 by crossed immunoelectrophoresis (2, 3). Crossed immunoelectrophoresis with absorption in situ was used by Syverson et al. to distinguish six cytoplasmic antigens unique to the mycelial or yeast phase of *C. albicans* (21). Such extracts provide information about the antigens which are under the surface.

In a previous study using crossed immunoelectrophoresis and soluble cytoplasmic extracts, we confirmed that *C. albicans* serotype A shared more than 70% of its antigens with *C. tropicalis* (10). It was possible, however, to demonstrate specific soluble antigens of *C. tropicalis* by using crossed immunoelectrophoresis performed with the *C. tropicalis* cell wall antiserum. In the present work, cytoplasmic soluble extracts of *C. albicans* serotype A and serotype B reference strains were prepared and studied by various quantitative immunoelectrophoretic methods. Crossed-line immunoelectrophoresis and crossed-line immunoelectrophoresis with absorption of the antibodies in situ were the methods of choice for these complex antigenic preparations and allowed us to demonstrate four specific soluble antigens unique to *C. albicans* serotype A extract.

MATERIALS AND METHODS

C. albicans strains 3153 group A and 3156 group B, used throughout these studies, were supplied by E. Drouhet, Institut Pasteur, Paris, France. For preparation of the antigen (1, 3, 9), a 10-g amount of a washed 48-h culture from Sabouraud medium (28°C) was suspended in 10 ml of 0.154 M NaCl and homogenized in a Braun cell homogenizer (MSK) at 2,800

rpm for 120 s with 50 g of glass beads (0.45 to 0.50 mm); liquid CO_2 was used as a coolant. The homogenate was decanted from the glass beads and centrifuged for 60 min at 105,000 × *g* and 4°C in a Beckman LS65 ultracentrifuge (SW50.1 rotor). The clear supernatant fluid was membrane filtered and stored at −20°C. The protein concentrations of group A and B antigens were determined to be, respectively, 11.7 and 12.1 g/liter by the Coomassie/Brilliant blue G method (20).

The antigens of *C. albicans* groups A and B were compared by using several quantitative immunoelectrophoretic techniques. Crossed immunoelectrophoresis and tandem crossed immunoelectrophoresis were carried out according to Weeke (27) and Kroll (14), respectively, with 1% agarose gel (Indubiose A 37, Industrie Biologique Française, Clichy, France) in tris(hydroxymethyl)aminomethane barbital buffer (pH 8.6; ionic strength, 0.02). The first-dimension electrophoresis of the *Candida* antigens (10 μl) was performed at 14°C, applying 10 V/cm for 70 min. The second-dimension electrophoresis was run at 14°C, applying 3 V/cm for 18 h through a gel containing 10 μl of goat anti-*C. albicans* group A serum (Institut Pasteur Production, Paris) per cm^2. In the first-dimension electrophoresis, the plates measured 11 by 9 cm and the thickness of the gel was 0.15 cm. In the second-dimension electrophoresis, they were 10 by 10 cm and 0.1 cm, respectively.

Line immunoelectrophoresis was performed as described by Kroll (15). Each antigen (60 μl) was mixed with 0.6 ml of 1% agarose and poured to obtain a 1- by 5- by 0.13-cm gel on a 10- by 10-cm glass plate. The antibody containing-gel was placed at 1 cm of the two adjoining antigen gels and measured 6 by 10 by 0.10 cm. The antibody concentration used and electrophoresis conditions were as described for the second dimension of crossed or tandem crossed immunoelectrophoresis.

Crossed-line immunoelectrophoresis was performed as described by Kroll (16). The first dimension was done as for crossed immunoelectrophoresis, with 10 μl of *C. albicans* group A antigen. A 10- by 1.5-cm slab was transferred to a coated glass plate (10 by 10 cm). A 10- by 2.3- by 0.13-cm blind gel was poured along the first-dimensional electrophoresis gel. After solidification, an 8- by 0.5-cm gel strip was cut out of the blind gel and replaced with gel containing antigen. This intermediate gel contained 50 μl (10%) of group A or group B *C. albicans* antigen. The gel containing antibody was formed by pouring 6.5 ml of agarose with *C. albicans* group A antiserum onto the remainder of the glass plate. The antiserum concentrations and the second-dimension electrophoresis conditions were as described for crossed or tandem crossed immunoelectrophoresis.

Crossed-line immunoelectrophoresis with absorption of antibodies in situ (5) was performed like crossed-line immunoelectrophoresis, but the amount of the *C. albicans* group B antigen in the gel was increased about 12-fold. This was achieved by using *C. albicans* group B antigen concentrated threefold by dialysis and poured to form an 8- by 1- by 0.13-cm-wide gel containing 20% antigen. All of the other conditions were the same as for crossed-line immunoelectrophoresis.

The nonprecipitated proteins were always removed by pressing the gels (26). After drying, the plates were stained for 10 min in 0.5% (wt/vol) Coomassie brilliant blue R in ethanol-glacial acetic acid-water (45:10:45). Excess dye was removed by repeated washings in ethanol-glacial acetic acid-water (25:10:45) destainer.

RESULTS

Crossed immunoelectrophoresis of group A and group B *C. albicans* antigens against anti-*C. albicans* group A serum showed, respectively, 60 and 58 individual precipitates (Fig. 1 & 2). Qualitative analysis showed that two precipitation peaks, present with the A antigen, seemed to be absent with the B antigen.

Figure 3 shows the result of tandem crossed immunoelectrophoresis, revealing antigenic similarities between group A and B *C. albicans*. In this procedure, the fusion of identical precipitin lines makes possible a direct qualitative comparison of different crossed immunoelectrophoresis patterns (14). Seven double peaks were observed in the complex pattern (Fig. 3). Comparison of the two extracts by line immunoelectrophoresis is shown in Fig. 4. About 20 different precipitation lines could be enumerated in each individual pattern, and one line, obtained with the A antigen, did not seem to be continued in the adjoining B-antigen pattern.

Figure 5 shows homologous crossed-line immunoelectrophoresis with group A antigen in the well of the first-dimension electrophoresis, group A antigen in the intermediate gel, and anti-A serum in the upper gel for the second

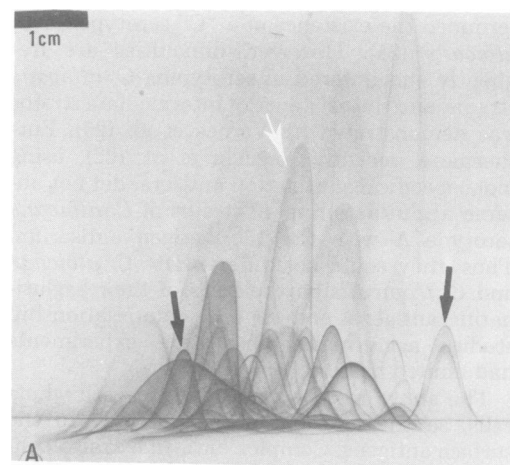

FIG. 1. *Crossed immunoelectrophoresis of C. albicans group A antigen and anti-A serum. Anodes are at the right and at the top. Black arrows indicate two precipitation peaks absent with the B antigen in Fig. 2 but found to be common constituents in Fig. 7. The white arrow indicates the most important specific antigen of C. albicans group A.*

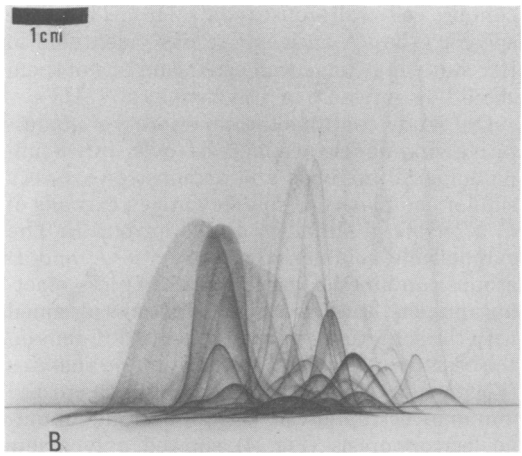

FIG. 2. *Crossed immunoelectrophoresis of C. albicans group B antigen and anti-A serum. Anodes are the right and at the top.*

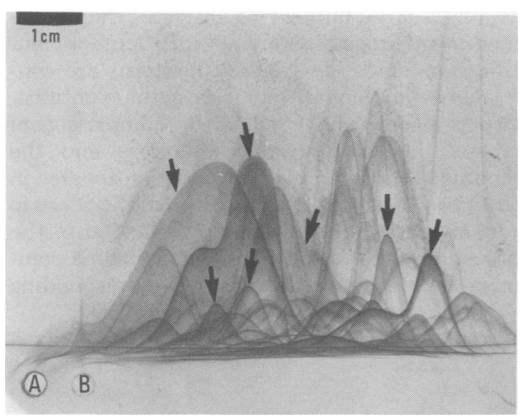

FIG. 3. *Tandem crossed immunoelectrophoresis comparing extracts of C. albicans group A and group B, using anti-A serum. Seven double peaks, indicated by arrows, show common antigens.*

dimension. Many peaks observed in crossed immunoelectrophoresis (Fig. 1) could be identified and were fused with a corresponding line. The result obtained in the same experiment but with group B antigen in the intermediate gel is shown in Fig. 6. Many peaks were extended, with lines indicating common antigenic constituents. Some peaks, however, appeared to be isolated, indicating possible specific components of *C. albicans* group A. Finally, Fig. 7 shows the pattern of crossed-line immunoelectrophoresis with absorption of antibodies in situ obtained in experiments as in Fig. 6 but with the amount of *C. albicans* group B antigen in the intermediate gel increased about 12-fold. This was achieved with *C. albicans* group B antigen concentrated threefold by dialysis, the amount of B antigen in the gel strip increased, and the gel strip enlarged.

Some peaks observed in Fig. 6 disappeared and others were continuous, with a basic line which migrated anodically. Of the six possible specific components of the A antigen observed in Fig. 6, two migrated anodically and were continuous, with a line indicating common constituents. Four remaining precipitates, however, appeared in unchanged positions in Fig. 6 and 7, indicating specific components of group A antigen. The most concentrated peak of these four specific precipitates could be identified in heterologous crossed-line immunoelectrophoresis without absorption of antibodies (Fig. 6) and in homologous crossed-line immunoelectrophoresis (Fig. 5), as well as in the reference crossed-immunoelectrophoresis pattern of *C. albicans* group A antigen (Fig. 1).

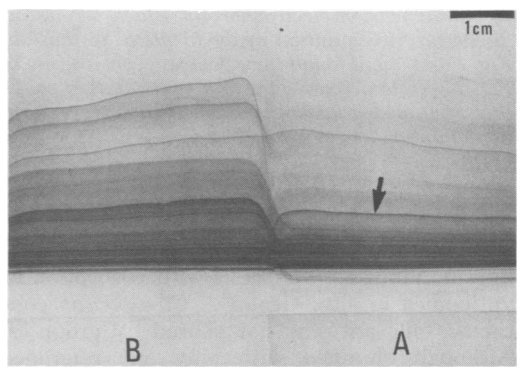

FIG. 4. *Line immunoelectrophoresis of C. albicans group A and B antigens, using anti-A serum. Arrow indicates a line obtained with the A antigen not continued in the adjoining B pattern.*

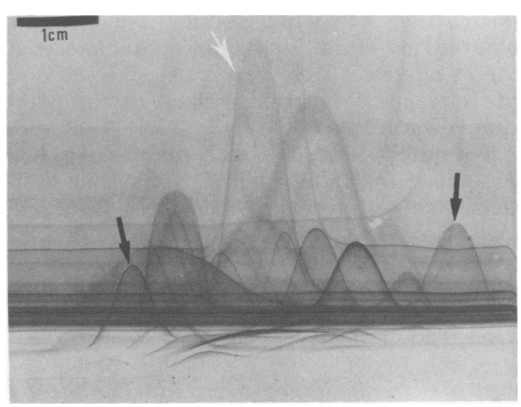

FIG. 5. *Crossed-line immunoelectrophoresis with group A antigen in the well for the first-dimension electrophoresis, group A antigen in the intermediate gel and anti-A serum for the second-dimension electrophoresis. Black and white arrows are as in the legend to Fig. 1.*

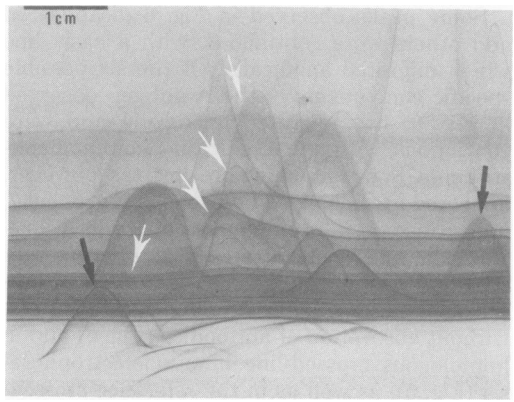

FIG. 6. *Crossed-line immunoelectrophoresis with group A antigen in the well for the first-dimension electrophoresis, group B antigen (normal concentration) in the intermediate gel and anti-A serum for the second-dimension electrophoresis. Black arrows indicate two precipitation peaks identical to those in Fig. 1 and 5 and found to be common constituents in Fig. 7. White arrows show four precipitation peaks that remained in unchanged positions after absorption of antibodies in situ in Fig. 7.*

DISCUSSION

Soluble cytoplasmic antigens specific to *C. albicans* group A were demonstrated by crossed-line immunoelectrophoresis with absorption of antibodies in situ. Group A *C. albicans* contained four antigens not shared by group B. Although obtained with only one reference strain of each group, the qualitative differences observed are supported by the study of Axelsen (3), who, in a comparison of eight *C. albicans* strains with strain B311 (group A) using crossed-line immunoelectrophoresis with adsorption of antibodies in situ, found no unique antigens and no reactions of partial identity. The patterns observed by crossed immunoelectrophoresis of the eight strains were very much like those of the reference B311 strain, with only some quantitative variations (3).

Our study, performed with complex extracts containing the water-soluble cytoplasmic antigens, enabled us to identify four antigens specific to *C. albicans* group A. Previous work performed with whole cells by agglutination-absorption showed that type A contains one antigen more than type B, further identified as antigen 6 by Tsuchiya et al. (24). Recently, we were able to demonstrate a specific soluble antigen of *C. tropicalis* not shared with *C. albicans* group A by using crossed immunoelectrophoresis performed with the *C. tropicalis* cell wall antiserum (10). Therefore, it is clear that soluble cytoplasmic antigens prepared by disruption of yeasts and removal of insoluble material may contain cell wall constituents (10). Thus, the specific group A antigens we have identified in this study may have wall origin and one of them should be antigen 6 of Tsuchiya et al. (24).

Our study confirms the superiority of quantitative immunoelectrophoresis over other immunological methods and demonstrates a very similar complexity in soluble antigen extracts of *C. albicans* of either group A or group B. The cytoplasmic soluble extracts of the A and B groups contained a large number of cross-reacting antigens; however, the two patterns obtained with the same antiserum were quite different on the basis of quantitative and qualitative analyses (Fig. 1 and 2). Nevertheless, tandem crossed immunoelectrophoresis (Fig. 3) and line immunoelectrophoresis (Fig. 4) showed only a few common antigens, and we would have incorrectly concluded at this stage of the work that *C. albicans* group A shared but few common constituents with group B. With extracts containing a large number of antigens, the above-mentioned procedures are not sufficient for identification, since the resulting patterns are confusingly complex (5). In our case, the identification problem can be solved by comparison of crossed line immunoelectrophoresis and the modification called absorption of antibodies in situ (5). The amount of B antigen in the gel strip was increased and the gel was enlarged in the procedure with absorption (Fig. 7). Some components in excess removed their corresponding

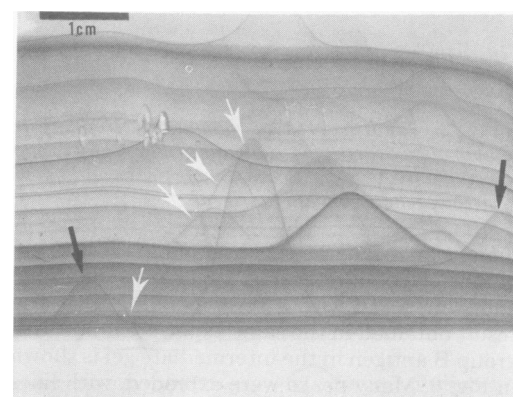

FIG. 7. *Crossed-line immunoelectrophoresis with absorption of antibodies in situ. Group A antigen was used in the first-dimension electrophoresis, group B antigen (12 times concentrated) in the intermediate gel and anti-A serum for the second-dimension electrophoresis. Compared with Fig. 6, black arrows show two precipitation peaks which changed their positions, indicating common constituents, and white arrows show four precipitation peaks in unchanged positions, indicating specific components of C. albicans group A.*

antibodies and disappeared. Other common components, present in the B antigen at lower concentrations, migrated anodically and many peaks connected with lines were revealed. Two of the six possible specific components appeared to be cross-reacting antigens, although they were absent in the crossed immunoelectrophoresis patterns of the B antigen (Fig. 2) and were not extended with a line in the crossed-line immunoelectrophoresis experiment with normal antigen concentrations (Fig. 5). As in other studies (1, 3, 9, 10, 13, 17-19), our results indicate that quantitative immunoelectrophoresis provides more detailed information than conventional immunoelectrophoresis regarding the number of immunologically reactive antigenic constituents and was found to be useful in evaluating the quality of various antigens. However, for taxonomic use in determining relationships between the organisms (4, 13, 17-19) or between two phases of the same organism (21) and in their identification, crossed immunoelectrophoresis, tandem crossed immunoelectrophoresis, line immunoelectrophoresis, and crossed-line immunoelectrophoresis may not be sufficient.

Hasenclever and Mitchell showed that type A contains at least one antigen more than type B (11), and it would be of interest to investigate the presence of soluble specific components in cytoplasmic extracts of type B. We are presently producing a reference anti-B polyspecific serum which should permit us to perform reverse experiments.

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