Antigenic Relationship Between American and African Isolates of Blastomyces dermatitidis as Determined by Immunoﬂuorescence

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The antigenic relationship between American and African isolates of Blastomyces dermatitidis was investigated through the use of the fluorescent antibody technique. Preliminary results suggest that the American and African isolates studies are not antigenically identical. The American isolates share antigens with the African ones; however, it appears that they also possess distinct antigens. Additional studies are necessary to confirm these findings and to draw any conclusion whether the African isolates represent a serotype(s) of B. dermatitidis or a distinct species.

Until recently blastomycosis was considered to be limited to the North American continent (1). However, over the past decade, autochthonous cases of blastomycosis have been described from the African continent (3–6). The etiologic agent in all of the African cases was identified as Blastomyces dermatitidis.

During this same period, African cultures that were considered to be B. dermatitidis and which had been submitted to the Developmental Mycology Section, Center for Disease Control, for conﬁrmation stained poorly or not at all with a fluorescent antibody reagent speciﬁc for the yeast-form of B. dermatitidis (7). This fluorescent antibody reagent had been prepared from antiglobulins to an American isolate of B. dermatitidis. These staining deﬁciencies posed a diagnostic problem and prompted the present investigation into the possibility that the African isolates tested were either serotypes of B. dermatitidis or possibly represented a new species of Blastomyces.

MATERIALS AND METHODS

Preparation of immune serum. Three albinino female rabbits, weighing 5 to 8 lbs., were immunized according to the method of Kaplan and Kaufman (7). One series of booster injections was given 1 week later for 3 days in the same dosage and via the same route as the initial series. Before immunization and again 7 days after the final injection, the animals were bled. The antigens were Formalin-killed (1.0%) yeast-form cells of B. dermatitidis prepared from an American isolate, no. B-414 (7), and an African isolate, no. B-834. Cells were grown in brain-heart infusion broth for 14 days at 37° C on a rotary shaker, killed with 1.0% buffered neutral Formalin, suspended in 0.5% buffered neutral Formalin, and adjusted to a no. 5 MacFarland nephelometric standard for immunization.

Preparation of fluorescein-labeled reagents. Preimmunization and immune globulins were obtained by fractionating the sera with half-saturated ammonium sulfate according to methods previously described (9). The globulins were conjugated with fluorescein isothiocyanate. The procedures for conjugation, removal of unreacted fluorescein, and adsorption with acetone-dried tissue powder for removal of tissue-staining factors were also described previously (9).

Preparation of staining material. Smears were made from aqueous suspensions of Formalin-killed (0.5% Formalin) yeast-form cells on etched ring slides, and were air-dried in a hot-air oven (45 °C). Isolates (Tables 1 and 2) were grown and harvested in the same manner as that used in preparing immunization antigens. The staining procedure used was previously described by Kaplan and Kaufman (7).

Adsorption of conjugates. Cross-staining factors were removed by adsorbing the fluorescein-labeled antiglobulins (conjugates) with cells of the immunization strain belonging to the heterologous group. Cells for adsorption were prepared in the same manner as the immunization antigens. Two volumes of conjugate were adsorbed with 1 volume of wet-packed cells at 37° C (water bath) for 2 h.

Visualization of staining. Stained preparations were examined with a Reichert Biozet microscope fitted with a cardioid dark-field condenser. A Reichert Fluorex unit fitted with an Osram HBO-200 high pressure mercury vapor lamp was used as the light source. A 442-1 American Optical interference filter (8) was used in combination with a GG-9 ocular filter. The intensity of staining was rated according to the
criteria of Cherry et al. (2) as follows: 4+, 3+, 2+, 1+, positive reactions, and +/-, 0, no reactions.

RESULTS

Staining properties of the unasorbed conjugates. One series of booster injections (1 week) was necessary to obtain a titer of antiglobulins which, when tagged with fluorescein isothiocyanate, would stain the immunization strain and other isolates of the homologous group 2 to 4+. The staining properties of the conjugates are summarized in Table 3. The unasorbed conjugates for each type (American and African) stained isolates of the homologous species as well as isolates of the heterologous species at levels ranging up to 4+. Occasionally, a few cells from some of the isolates were not stained (Table 3). Fluorescein-labeled preimmunization globulins did not stain any of the isolates.

The cross-reactivities of the unasorbed conjugates with organisms of other genera are presented in Table 4. Each reagent stained several of these fungi and, with few exceptions, at the same level of intensity as the homologous species. The conjugate for the American B. dermatitidis cross-stained more fungi of other genera than did the conjugate for the African B. dermatitidis. Of clinical significance is the fact that the African conjugate stained H. capsulatum var. duboisii, the agent of the African large-form histoplasmosis, whereas the American conjugate did not.

Staining properties of the adsorbed conjugates. The staining properties of the adsorbed American and African conjugates are presented in Tables 3 and 4. The African conjugate, when adsorbed with American B. dermatitidis cells (yeast-form), lost its capacity for staining both the African and American isolates. In contrast, the American conjugate, when adsorbed with African B. dermatitidis yeast-form cells, retained its capacity for staining the American isolates, but lost its staining capacity for the African isolates, with the exception of strain

| CDC* identification no. | Country where culture was isolated | Further information as to source | Other identification no. | Donor |
|-------------------------|-----------------------------------|---------------------------------|--------------------------|-------|
| B 832                   | Uganda                            | King                            | 6071                     | H. F. Hasenclever, National Institute for Allergy and Infectious Diseases |
| B 833                   | South Africa                      | Lurie                           | 6066                     | F. Gatti |
| B 834                   | Mozambique                        | Magalhanes                      | 6074                     | E. S. McDonough, Marquette University |
| B 838                   | Mozambique                        | Magalhanes                      | 766                      | McDonough, Marquette Univ. |
| B 781                   | Zaire                             | F. Gatti                        | 767                      | McDonough, Marquette Univ. |
| B 1562                  | Rhodesia                          | London School of Tropical Medicine and Hygiene | 4047                     | McDonough, Marquette Univ. |
| B 1563                  | South Africa                      | London School of Tropical Medicine and Hygiene | 4048                     | McDonough, Marquette Univ. |
| B 1564                  | Morocco                           | Pasteur Institute               | 973                      | McDonough, Marquette Univ. |
| B 1565                  | Zaire                             | Vandelitte                      | ZN                       | McDonough, Marquette Univ. |
| B 1566                  | South Africa                      | Koornhof                         | 581                      | McDonough, Marquette Univ. |

* CDC, Center for Disease Control.

| CDC* identification no. | By whom submitted | Source |
|-------------------------|-------------------|--------|
| B-414                   | E. S. McDonough, Marquette University | Human, skin lesion |
| B-130                   | Adolph Bleir, Chester, Pa. | Patient resided in Lexington, Kentucky |
| 45-1247-71              | USAF Medical Center, Scott AFB, Ill. | Human |
| A-295                   | State Laboratory of Hygiene, Raleigh, N.C. | Human, sputum |
| 45-252-71               | State Dept. of Health, Phoenix, Ariz. | Human |
| 45-1229-71              | Jackson Memorial Hospital, Miami, Fla. | ? |
| 45-730-71               | Mississippi State Dept. of Health Laboratories, Jackson, Miss. | Human, gastric washings, sputum |
| 45-76-62                | University of Illinois, Urbana, Ill. | Human, pleural fluid |
| 45-1442-58              | Middle Tennessee T.B. Hospital, Nashville, Tenn. | Human |
| 497                     |                    |        |

* CDC, Center for Disease Control.
### Table 3. Staining properties of African and American isolates of B. dermatitidis

| Cultures         | Staining reactions with fluorescein-labeled globulins against |
|------------------|---------------------------------------------------------------|
|                  | African B. dermatitidis | American B. dermatitidis |
|                  | U* | Am | Pb | U  | Af | Pb |
| African B-834*   | 3-4+ | 0  | 0  | 0-2+ | 0  | 0  |
| African B-833    | 3-4+ | 0  | 0  | 0-2+ | 0  | 0  |
| African B-832    | 3-4+ | 0  | 0  | 0-3+ | 0  | 0  |
| African B-781    | 2-4+ | 0  | 0  | 0-2+ | 0  | 0  |
| African B-838    | 0-3+ | 0  | 0  | 0-2+ | 0  | 0  |
| African B-1562   | 3-4+ | 0  | 0  | 0-3+ | 0  | 0  |
| African B-1563   | 3-4+ | 0  | 1-2+ | 2-3+ | 0  | 0-2+ |
| African B-1564   | 4+  | 0  | 0  | 3-4+ | 0  | 2-3+ |
| African B-1565   | 0-4+ | 0  | 0  | 0-1+ | 0  | 0  |
| African B-1566   | 1-4+ | 0  | 1-2+ | 0-3+ | 0-1+ | 2-3+ |
| American B-414*  | 3-4+ | 0  | 0  | 2-4+ | 0-2+ | 0-2+ |
| American B-390   | 1-3+ | 0  | 0  | 2-4+ | 0-2+ | 0-2+ |
| American 45-1247-71 | 0-4+ | 0  | 0  | 0-3+ | 0-2+ | 0-2+ |
| American 45-252-71 | 0-4+ | 0  | 0  | 2-3+ | 0-1+ | 0  |
| American 45-1229-71 | 0-4+ | 0  | 0  | 2-4+ | 0-3+ | 0-2+ |
| American 45-370-71 | 2-4+ | 0  | 0  | 3-4+ | 0-2+ | 0-2+ |
| American 76-62   | 1-4+ | 0  | 0  | 3-4+ | 1-3+ | 0-3+ |
| American 1442    | 2-4+ | 0  | 0  | 1-3+ | 0-1+ | 0  |
| American A-295   | 1-4+ | 0  | 0  | 1-4+ | 0-1+ | 0-1+ |

*U, Unabsorbed fluorescein-labeled antiglobulins; Am, adsorbed (with American cells) fluorescein-labeled antiglobulins; Af, adsorbed (with African cells) fluorescein-labeled antiglobulins; Pb, adsorbed (with Paracoccidioides brasiliensis cells) fluorescein-labeled antiglobulins.

### Table 4. Staining properties of heterologous fungi

| Organism                  | Staining reactions with fluorescein-labeled globulins against |
|---------------------------|---------------------------------------------------------------|
|                           | African B. dermatitidis | American B. dermatitidis |
|                           | U* | Am |       | U  | Af |       |
| Candida albicans (2)*     | 0  | –  | 0     | –  | –  | –     |
| C. parapsilosis (2)       | 0  | –  | 0-2+  | –  | –  | –     |
| C. pseudotropicalis (2)   | 0  | –  | 1+    | 0  | –  | –     |
| C. stellatoidea (2)       | 0  | –  | 0     | –  | –  | –     |
| C. tropicalis (2)         | 0  | –  | 0-1+  | 0  | –  | –     |
| Cryptococcus albidus (2)  | 0  | –  | –     | 0  | –  | –     |
| C. diffuens (2)           | 0  | –  | 0-1+  | 0  | –  | –     |
| C. neoformans (2)         | 0  | –  | 0     | –  | –  | –     |
| C. terreus (2)            | 0  | –  | –     | –  | –  | –     |
| Geotrichum candidum (2)   | 1-4+ | 0  | 2-4+  | 0-4+ | 0-4+ | 0-4+ |
| Histoplasma capsulatum (2)| 0-3+ | 0  | 2-4+  | 0  | –  | –     |
| H. duboisii (2)           | 0-3+ | 0  | 0     | –  | –  | –     |
| Paracoccidioides brasiliensis (4) | 2-4+ | 0  | 1-3+  | 0-1+ | 0-1+ | 0-1+ |
| Sporothrix schenckii (2)  | 0  | –  | 0     | –  | –  | –     |
| Saccharomyces cerevisiae (1)| 0  | –  | 0     | –  | –  | –     |
| Trichosporon cutaneum (1) | 0  | –  | 0     | –  | –  | –     |
| Torulopsis glabratra (1)  | 0  | –  | 0     | –  | –  | –     |
| African B. dermatitidis (10)| 3-4+ | 0  | 0-4+  | 0  | –  | –     |
| American B. dermatitidis (9)| 0-4+ | 0  | 0-4+  | 0  | –  | 3-3+  |

*U, Unadsorbed fluorescein-labeled antiglobulins; Am, adsorbed (with American cells) fluorescein-labeled antiglobulins; Af, adsorbed (with African cells) fluorescein-labeled antiglobulins.

*a Number of strains used: 0, 1, 2, 3, 4, – intensity of staining; 0-1, 2, etc. indicate that cells of all isolates stained in the range indicated.

+c Essentialy negative; rare cells stained as brightly as 4+.

+3 Three isolates did not stain; cells of one isolate stained in the range indicated.
B-1566. Those heterologous fungi that were stained by the unadsorbed African conjugate were not stained by the adsorbed reagent. With two exceptions, *Geotrichum candidum* and *Paracoccidioides brasiliensis*, the same was observed with the adsorbed conjugate for the American *B. dermatitidis*. The level of staining in these two exceptions was of a low order.

The African conjugate, when adsorbed with the yeast-form cells of *P. brasiliensis*, lost its capacity for staining the American isolates and all but two of the African isolates. In contrast, the American conjugate, when adsorbed with the same cells, still stained seven of the nine American isolates as well as four of the 10 African isolates.

The African and American conjugates were not adsorbed with their respective homologs. Prior experience has shown that such adsorptions yield a reagent which has lost its staining capacity.

**DISCUSSION**

The results of this preliminary study, as summarized in Table 3, provide evidence that the African isolates of *B. dermatitidis* studied are antigenically different from the American isolates. Although both isolate groups share antigens, it appears that the American isolates also have distinct antigens.

The American isolates appear to be antigenically more complex. The conjugate prepared from African *B. dermatitidis* antiglobulins, when adsorbed with cells of the American variety, lost capacity for staining. On the other hand, when the conjugate that had been prepared from American *B. dermatitidis* antiglobulins was adsorbed with cells of the African variety, it retained its capacity for staining—but only for the American isolates.

A similar phenomenon was observed when the conjugates for the African and American varieties of *B. dermatitidis* were adsorbed with yeast-form cells of *P. brasiliensis*. With two exceptions, none of the *B. dermatitidis* isolates, American or African, were stained by the adsorbed African conjugate. On the other hand, the American conjugate stained seven out of nine of the American isolates and four out of ten African isolates. This, then, provides additional evidence that the American isolates are antigenically more complex than the African isolates. These findings also suggest that the African isolates of *B. dermatitidis* are more closely related antigenically to *P. brasiliensis* than to the American isolates of *B. dermatitidis*. To prove closeness of these antigenic relationships, it would, of course, be necessary to prepare conjugates from *P. brasiliensis* antiserum and perform cross-adsorptions.

It is not possible on the basis of these preliminary results to draw definitive conclusions as to whether African strains represent a distinct serotype(s) of *B. dermatitidis*, or whether they might even warrant a separate species designation. Further, investigations using additional African and American strains for antibody production and cross-adsorption, as well as mating studies, are necessary before these questions are resolved.

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