

Monoclonal Antibody 13F1 Produces Annular Immunofluorescence Patterns on *Cryptococcus neoformans* Serotype AD Isolates

Cryptococcus neoformans isolates have been grouped serologically into five serotypes known as A, D, AD, B, and C. Recently, a proposal to separate serotype A and D isolates into two varieties, known as *grubii* and *neoformans*, respectively, was made (4). Of the five serotypes, the AD type is probably the least studied. Restriction fragment length polymorphism, multilocus enzyme electrophoresis, and random amplified polymorphic DNA analyses reveal that AD isolates comprise a discrete group that can be distinguished from both serotypes A and D (2, 7, 9).

Monoclonal antibody (MAb) 13F1 is a murine immunoglobulin M (IgM) that binds to the capsular glucuronoxylomannan (3). Indirect immunofluorescence (IF) staining of serotype A and D strains produces annular and punctate patterns, respectively (3, 8). Differences in MAb 13F1 IF binding have been used to support the proposal to separate these serotypes into two varieties (4). In the present study, we investigated the IF pattern of MAb 13F1 for 12 serotype AD isolates which had been collected from Atlanta, Ga.; San Francisco, Calif.; Houston, Tex.; and the state of Alabama as part of the Centers for Disease Control and Prevention cryptococcal active surveillance project (1). IF was done as described elsewhere (3).

All isolates produced annular IF with MAb 12A1, showing that this IgM does not discriminate among serotypes A, D, and AD. In contrast, MAb 13F1 produced a qualitatively different IF pattern only with serotype D isolates. Of 15 AD isolates, all except one produced annular IF patterns with MAb 13F1. For this isolate there was a mixed IF pattern. These results show that MAb 13F1 IF patterns do not discriminate between the A and AD serotypes. The finding that MAb 13F1 binding to AD isolates produced annular IF provides additional support for the conclusion that the punctate IF pattern with MAb 13F1 is highly predictive of serotype D classification (3). The production of a punctate IF pattern with MAb 13F1 has been proposed as a characteristic distinguishing between the varieties *grubii* and *neoformans* (4). The finding that MAb 13F1 discriminates between serotypes AD and D supports its usefulness in identifying variety *neoformans* (serotype D) isolates. The specificity of MAb 13F1 is different from that of the serotype D-specific MAb CRND-8 described by Ikeda et al., which reacted with serotypes D and AD but not serotype A (6). Chemical analysis of the capsular polysaccharide of serotype AD strains shows that it is very similar to that of serotypes A and D but differs primarily in the ratio of substituted to unsubstituted mannose residues (5). The finding of Ikeda et al. that MAb CRND-8 discriminated between serotypes AD and A (6) combined with our finding that MAb 13F1 discriminates between AD and D provides support for the proposal that AD does represent a fifth *C. neoformans* serotype.

REFERENCES

1. Brandt, M., L. C. Hutwagner, L. A. Klug, W. S. Baughman, D. Rimland, E. A. Graviss, R. J. Hamill, C. Thomas, P. G. Pappas, A. L. Reingold, and R. W. Pinner. 1996. Molecular subtype distribution of *Cryptococcus neoformans* in four areas of the United States. *J. Clin. Microbiol.* **34**:912-917.
2. Brandt, M. E., L. C. Hutwagner, R. J. Kuykendall, R. W. Pinner, and The Cryptococcal Disease Active Surveillance Group. 1995. Comparison of multilocus enzyme electrophoresis and random amplified polymorphic DNA analysis for molecular subtyping of *Cryptococcus neoformans*. *J. Clin. Microbiol.* **33**:1890-1895.
3. Cleare, W., and A. Casadevall. 1998. The different binding patterns of two immunoglobulin M monoclonal antibodies to *Cryptococcus neoformans* serotype A and D strains correlate with serotype classification and differences in functional assays. *Clin. Diagn. Lab. Immunol.* **5**:125-129.
4. Franzot, S. P., I. F. Salkin, and A. Casadevall. 1999. *Cryptococcus neoformans* var. *grubii*: separate varietal status for *Cryptococcus neoformans* serotype A isolates. *J. Clin. Microbiol.* **37**:838-840.
5. Ikeda, R., H. Matsuyama, A. Nishikawa, T. Shinoda, and Y. Fukazawa. 1991. Comparison of serological and chemical characteristics of capsular polysaccharides of *Cryptococcus neoformans* var. *neoformans* serotype A and *Cryptococcus albidus* var. *albidus*. *Microbiol. Immunol.* **35**:125-138.
6. Ikeda, R., S. Nishimura, A. Nishikawa, and T. Shinoda. 1996. Production of agglutinating monoclonal antibody against antigen 8 specific for *Cryptococcus neoformans* serotype D. *Clin. Diagn. Lab. Immunol.* **3**:89-92.
7. Kohno, S., A. Varma, K. J. Kwon-Chung, and K. Hara. 1994. Epidemiology studies of clinical isolates of *Cryptococcus neoformans* of Japan by restriction fragment length polymorphism. *J. Jpn. Assoc. Infect. Dis.* **68**:1512-1517.
8. Nussbaum, G., W. Cleare, A. Casadevall, M. D. Scharff, and P. Valadon. 1997. Epitope location in the *Cryptococcus neoformans* capsule is a determinant of antibody efficacy. *J. Exp. Med.* **185**:685-697.
9. Viviani, M. A., H. Wen, A. Roverselli, R. Caldarelli-Stefano, M. Cogliati, P. Ferrante, and A. M. Tortorano. 1997. Identification by polymerase chain reaction fingerprinting of *Cryptococcus neoformans* serotype AD. *J. Med. Vet. Mycol.* **35**:355-360.

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