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# Serological Analysis of Dermatophyte Isolates with Monoclonal Antibodies Produced Against *Microsporum canis*

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**Hybridoma cells produced by fusing myeloma cells with spleen cells from mice immunized with a soluble antigen of *Microsporum canis* yielded 30 antibody-producing clones. Six of these clones, propagated as ascites tumors in mice, showed two different types of monoclonal antibodies. The type 1 monoclonal antibody reacted with 17 heterologous and 10 homologous dermatophyte antigens. Type 2 monoclonal antibodies were unable to precipitate three antigens from different isolates of *M. canis*, thus suggesting the occurrence of different serotypes within the species.**

Dermatophytes are classified in three genera and 50 species essentially on the basis of their gross colonial characteristics and micromorphology. Pleomorphism and atypical sporulation have made medical mycologists aware of the need to evaluate other criteria to rapidly and reliably identify dermatophyte species. Although there now exist biochemical methods, serological tests, involving antisera produced against reference antigens, remain the major alternate laboratory procedure for the differentiation of some dermatophyte species (1, 2, 5). The problem with these assays arise from the fact that polyvalent antisera prepared against the authentic strains of the dermatophytes cross-react in serological tests, and adsorption procedures lead to the total loss of reactivity. Hybridoma technology offers the potential to solve these problems, and in this study, we report the production of monoclonal antibodies to soluble antigens of *Microsporum canis* Bodin.

**Cultures.** The fungal isolates used in the study were from our collection (Università Cattolica del Sacro Cuore [UCSC]) or were kindly furnished by Libero Ajello, Division of Mycotic Diseases, Centers for Disease Control (CDC), Atlanta, Ga. (Table 1).

**Reference antigens.** *M. canis* CDC B2094, was the strain used for the production of the reference antigen. It was derived from a Merthiolate-treated solution (1:5,000) and concentrated 50× (3) by a PM10 membrane in a TCF10A Ultrafiltrator cell (Amicon Corp., Lexington, Mass.) obtained from many Sabouraud-Dextrose agar slant cultures of different ages.

Serological analyses had previously shown that the antigen was comparable to one obtained via an acetone-treated filtrate.

**Mouse immunization.** BALB/c mice were immunized according to the following protocol. A 0.1-ml amount of the reference antigen mixed with 0.1 ml of incomplete Freund adjuvant was injected intraperitoneally once a week for 1 month. At 3 days before fusion, the mice were primed (intraperitoneal injection) with the soluble reference antigen (0.1 ml).

**Cells and media.** The NS1 cell line was kindly furnished by Lenore Pereira, Department of Health, Virology Laboratory, Berkeley, Calif. It was derived from a BALB/c myeloma line. The NS1 cell line and the hybrids derived from the fusion of NS1 cells with BALB/c mouse spleen cells were grown in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% fetal bovine serum.

**Hybridization and selection of antibody-producing hybrids.** After 3 days of booster injections, the hyperimmune mice

TABLE 1. Serological analysis of dermatophyte isolates with monoclonal antibodies to *M. canis* CDC B2094 as judged by immunodiffusion

| Fungus isolates | Reactivity[a] of monoclonal antibodies | |
|---|---|---|
| | Type 1 | Type 2 |
| *M. canis* | | |
| CDC B2094 | + | + |
| UCSC 0 | + | + |
| UCSC 1 | + | + |
| UCSC 2 | + | − |
| UCSC 4 | + | − |
| UCSC 6 | + | + |
| UCSC 8 | + | − |
| UCSC 10 | + | + |
| UCSC 11 | + | + |
| UCSC 12 | + | + |
| UCSC 14 | + | + |
| *Epidermophyton floccosum* CDC B3807 | + | + |
| *Microsporum audouinii* CDC B3800 | + | + |
| *M. cookei* CDC B3803 | + | + |
| *M. distortum* CDC B2174 | + | + |
| *M. equinum* CDC B2699 | + | + |
| *M. ferrugineum* CDC 83-056097 | + | + |
| *M. gallinae* CDC B3801 | + | + |
| *M. gypseum* CDC B3816 | + | + |
| *M. nanum* CDC B3815 | + | + |
| *M. persicolor* CDC B1923 | + | + |
| *Trichophyton mentagrophytes* var. *erinacei* CDC B1865 | + | + |
| *T. mentagrophytes* var. *mentagrophytes* CDC 6209 | + | + |
| *T. mentagrophytes* var. *quinckeanum* CDC B2331 | + | + |
| *T. rubrum* CDC B3806 | + | + |
| *T. soudanense* CDC 83-048430 | + | + |
| *T. tonsurans* CDC B3810 | + | + |
| *T. violaceum* CDC B3610 | + | + |

[a] +, Reactivity; −, loss of reactivity.

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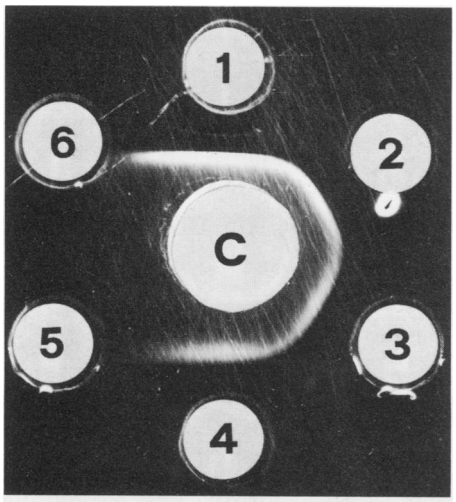


FIG. 1. Immunodiffusion test of *M. canis* isolates with monoclonal antibodies. Well contents: c, type 2 monoclonal antibody ascites fluid; 1 and 4, *M. canis* CDC B2094 antigen; 2, *M. canis* UCSC O antigen; 3, *M. canis* UCSC 1 antigen; 5, *M. canis* UCSC 2 antigen; 6, *M. canis* UCSC 4 antigen.

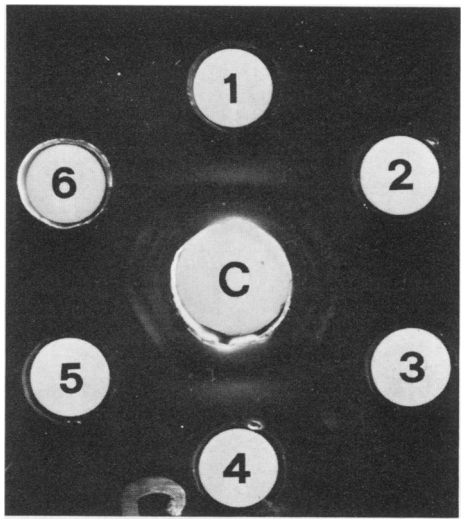


FIG. 2. Immunodiffusion test of *M. canis* isolates with rabbit polyvalent antiserum. Well contents: c, rabbit polyvalent antiserum; 1 and 4, *M. canis* CDC B2094 antigen; 2, *M. canis* UCSC O antigen; 3, *M. canis* UCSC 1 antigen; 5, *M. canis* UCSC 2 antigen; 6, *M. canis* UCSC 4 antigen.

were sacrificed, and their spleens were removed. The spleen cells were fused to an equal number of NS1 cells by polyethylene glycol 1000 (Sigma Chemical Co., St. Louis, Mo.). The cell culture was subjected to a 1-month selection regime by adding hypoxanthine, aminopterin, and thymidine (Flow Laboratories, Irvine, Ayrshire, United Kingdom) to the culture medium. For screening the hybrids (the cells producing the desired antibody) the hybrid fluids were tested by enzyme-linked immunoassay (6). Hybridoma cultures secreting the desired antibody were propagated and cloned by limiting dilution in the presence of mouse thymocytes. Clones continuing to produce the antibody were expanded. The monoclonality of the antibody produced by the various clones was ascertained by testing them by immunodiffusion against rabbit anti-mouse immunoglobulin antisera (Miles Laboratories, Elkhart, Ind.). For producing ascites fluid from which concentrated immunoglobulins could be obtained, an intraperitoneal injection of $10^7$ hybridoma cells was administered to Pristane-treated (Sigma Chemical Co.) (0.5 ml intraperitoneally) syngeneic BALB/c mice. The clones were frozen at −70°C and then maintained in liquid nitrogen.

**Serological tests.** An enzyme-linked immunoassay used for selecting the antibody-producing hybrids. To detect the reactivity of monoclonal antibodies in the ascites fluid to the homologous or heterologous antigens, an agar gel-double diffusion procedure was used (4).

Thirty clones were obtained from the fusion of NS1 myeloma cells with spleen cells from mice immunized with the reference antigen of *M. canis*. Monoclonal antibodies of the immunoglobulin G class were produced. Antibody-rich ascitic fluids produced from four of the six expanded clones (type 1) reacted in immunodiffusion either with all of the heterologous or homologous dermatophyte antigens. The other two clones (type 2) did not react with the antigens obtained from three different isolates of *M. canis* (Fig. 1) (Table 1). The antigens of the same three *M. canis* isolates that were negative with the type 2 monoclonal antibody regularly reacted with their homologous rabbit polyvalent antiserum (Fig. 2).

Hybridoma technology appears to offer high potential in the immunoidentification of fungi. In our experience, type 2 monoclonal antibody distinguished the different isolates of *M. canis*. This suggests the possibility that different serotypes may exist within the species. This approach could be used for both rapid immunoidentification of different species of morphologically related pathogenic fungi and the evaluation of antigenic heterogeneity (serotypes) in fungal populations other than *M. canis*.

The study of the reactivity of monoclonal antibodies with glycoprotein antigens which have been electrophoretically separated and transferred to nitrocellulose strips will yield, moreover, definitive information concerning the type specificity of the antibody and the nature of the glycoproteins. Such a study is presently under way at our institute.

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