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Antigenic Variation within Serotypes of *Cryptococcus neoformans* Detected by Monoclonal Antibodies Specific for the Capsular Polysaccharide

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The serotype-specific polysaccharide was isolated from four to seven strains of each of the four major serotypes of *Cryptococcus neoformans*. Reactivities of polysaccharides with seven monoclonal antibodies raised against polysaccharides of serotypes A, B, C, and D were assessed by an enzyme-linked immunosorbent assay and double immunodiffusion. The results indicated that there is heterogeneity within all four serotypes in the expression of epitopes reactive with monoclonal antibodies.

Serotypes A, B, C, and D represent the major types of cryptococcal polysaccharide. Several studies have suggested that variations in the antigenic structures of cryptococcal polysaccharides are not limited to four serotypes (2, 3, 6, 7, 9). We reported the production of monoclonal antibodies (MAbs) reactive with epitopes shared by two or more serotypes (5). In the present study we used MAbs reactive with shared epitopes to determine whether variations in reactivities with the MAbs occur within the four major serotypes.

Cryptococcus neoformans 24064 (serotype A), 24065 (serotype B), 24066 (serotype C), and 24067 (serotype D, originally NIH52) were obtained from the American Type Culture Collection, Rockville, Md. K. J. Kwon-Chung and J. E. Bennett generously provided serotype A strains 104, 271, 288, 289, and 371; serotype B strains 182, 435, 444, 1237, 1238, and 1317; serotype C strains 191, 917, and 1134; and serotype D strains 52, 529, 3501, and 3502.

Polysaccharides were isolated from the supernatant fluids of each strain as described previously (4). The immunoreactivity of each polysaccharide was determined in an enzyme-linked immunosorbent assay (ELISA) in which poly-L-lysine-treated plates were coated with each polysaccharide and the reactivity was assessed with serial dilutions of each MAb (5). The MAb concentration before dilution was 2 mg/ml. The substrate for the ELISA was 3,3′,5,5′-tetramethylbenzidine (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Md.), which was used in accordance with manufacturer directions. The optical density was read at 450 nm (OD_{450}). Precipitation of polysaccharides (2 mg/ml) by MAbs (2 mg/ml) was determined by double immunodiffusion in agar (5). Any assay which produced a reproducible precipitin line was scored as positive.

Hybridomas were produced by fusion of Sp/2.0-Ag14 myeloma cells and spleen cells from mice immunized with polysaccharides coupled to sheep erythrocytes. MAbs 302, 439, and 1255 have been described previously (5). MAbs 339, 386, 471, and 3C2 were produced in an identical manner by immunization, respectively, with polysaccharides from strains 24065, 24067, 24064, and 24066.

Three patterns were observed by the ELISA. Negative reactions consistently produced an OD_{450} of <0.2 at a 1/200 dilution of the MAb. A second pattern, referred to as full reactivity, was observed when the reaction of an MAb with a polysaccharide produced an OD_{450} of >1.5 at dilutions of 1/3,200 or greater. This pattern is illustrated in Fig. 1 by MAb 3C2 and polysaccharide from strain 104. Assays producing a maximal OD_{450} of >1.5 over several antibody dilutions are identified in Table 1 as having a ++ result. A third pattern, referred to as partial reactivity, was observed when the reaction of an MAb with a polysaccharide produced a reproducible OD_{450} of >0.2 but a maximal OD_{450} of <1.5. Typically, the maximal observable OD_{450} was between 0.5 and 1.2 and was identical over several antibody dilutions. This pattern is illustrated in Fig. 1 by polysaccharide from strain 191. Assays producing partial reactivity are identified in Table 1 as having a + result. The key difference between full and partial reactions was the maximal OD_{450}.

A limited availability of epitopes is a likely explanation for polysaccharides that produced partial reactions. This could be due to poor binding by the polysaccharide to the solid phase. Alternatively, the partial reaction could be due to restricted expression of the epitope reactive with the MAb. It is unlikely that poor binding explains the partial reactivity patterns because each of the polysaccharides producing a partial reaction with one MAb also produced a full reaction with at least one other MAb. Thus, restricted expression of

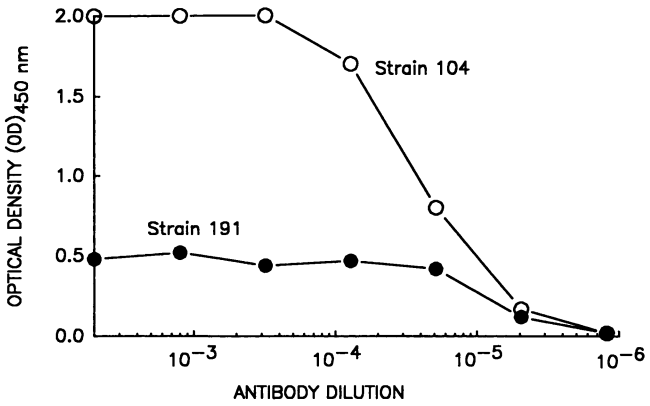

FIG. 1. Reactivity of polysaccharides from *C. neoformans* 104 and 191 with MAb 3C2 in the ELISA. The initial concentration of the MAb was 2 mg/ml.

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TABLE 1. Comparison of the reactivity of cryptococcal polysaccharides with MAbs in immunodiffusion (ID) and the ELISA

Serotype and strain	Reactivity with the following MAb:													
	302		386		339		1255		439		471		3C2	
	ID	ELISA	ID	ELISA	ID	ELISA	ID	ELISA	ID	ELISA	ID	ELISA	ID	ELISA
D														
3501	−	++	−	−	−	++	−	+	−	++	−	++	+	+
529	+	−	+	−	+	++	+	++	+	++	+	+	+	−
52	+	−	+	−	+	++	+	++	+	+	+	+	+	−
3502	+	++	+	−	+	++	+	++	+	++	+	+	+	+
A														
104	+	++	+	−	+	++	+	++	+	++	+	++	+	++
288	+	+	+	−	+	++	+	++	+	++	+	++	+	++
371	+	+	+	−	+	++	+	++	+	++	+	++	+	++
289	−	+	+	−	+	++	+	++	+	++	+	++	+	++
271	−	−	−	−	+	++	+	++	+	++	+	++	+	++
B														
444	−	−	+	−	+	++	+	++	+	++	+	++	+	++
24065	−	−	−	−	+	+	+	++	+	++	+	++	+	+
1238	−	−	−	−	+	++	+	++	+	++	+	+	+	+
1237	−	−	−	−	+	+	+	+	+	+	+	++	+	+
1317	−	−	−	−	+	+	+	+	+	++	+	++	+	+
182	−	−	−	−	+	+	+	+	+	++	+	++	+	+
435	−	−	−	−	+	+	+	++	+	+	+	++	+	++
C														
917	−	−	+	−	+	++	+	++	+	++	+	++	+	++
1134	−	−	+	−	+	++	+	++	+	++	+	++	+	++
24066	−	−	−	−	−	+	−	+	+	++	+	++	+	+
191	−	−	−	−	−	+	−	+	−	++	−	++	+	+

an epitope is the most likely explanation for the partial reactions.

Results of the ELISA and immunodiffusion (Table 1) revealed distinct reactivity patterns within all four serotypes. The greatest variability was observed within serotype D. Polysaccharide from strain 3501 differed from those of the remaining serotype D strains because it did not precipitate with any antibody except MAb 3C2. Serotype D strains 529 and 52 differed from the remaining serotype D strains because they produced negative ELISA results with MAb 302 and MAb 3C2. Serotype A strain 271 differed from the remaining serotype A strains because it failed to react with both MAb 302 and MAb 386, a pattern more like those of the serotype B and serotype C isolates. Strain 271 produced a negative reaction on CGB agar, the reaction expected of serotype A or D (8). In contrast, polysaccharide from serotype B strain 444 produced a precipitin line with MAb 386 and had ELISA reactions with MAb 339 and MAb 3C2 similar to those observed with polysaccharides from serotype A strains. Strain 444 produced a positive reaction on CGB agar, a result consistent with a serotype B or C isolate (8). Serotype C strains 24066 and 191 differed from the remaining strains of serotype C in failing to produce precipitin bands with MAb 386, MAb 339, and MAb 1255. In addition, polysaccharides from strains 917 and 1134 produced full reactions with MAb 339, MAb 1255, and MAb 3C2, while polysaccharides from strains 24066 and 191 produced partial reactions.

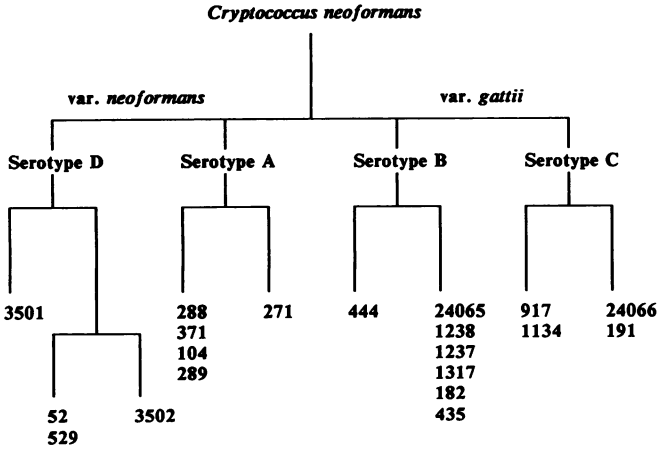

FIG. 2. Relationships among polysaccharides isolated from strains of *C. neoformans*.

MAb 386 was reactive in immunodiffusion with at least one isolate of each serotype but also failed to react with at least one isolate of each serotype. This result suggests the presence of an epitope that is highly variable in its expression. Alternatively, it is possible that a second acidic polysaccharide which is reactive with MAb 386 is produced by some isolates of *C. neoformans*. This latter possibility is supported by the report that two apparently acidic polysaccharides are produced by serotype B isolate 444 (1). This is one of the strains producing polysaccharide reactive with MAb 386.

MAb 386 did not produce any positive results in the ELISA, suggesting that the binding of polysaccharides to plates coated with poly-L-lysine altered or distorted the manner in which the epitope was presented. Alternatively, the binding of cryptococcal polysaccharide to the poly-L-lysine-coated plates could occur via the carboxyl group. If

the epitope recognized by MAb 386 includes the carboxyl group, its reactivity in an ELISA could be markedly altered.

Our results indicate that there are distinct subsets within each serotype of *C. neoformans*. A conservative representation of these subsets is summarized in Figure 2. It is quite possible that there are additional subsets. A definitive assignment of isolates to subsets will require an examination of additional strains with these and other MAbs and corresponding structural studies to identify structural correlates of the subsets.

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