

Sensitivity of Sandwich Enzyme-Linked Immunosorbent Assay for *Cryptococcus neoformans* Polysaccharide Antigen Is Dependent on the Isotypes of the Capture and Detection Antibodies

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Immunoglobulin M (IgM) and IgG1 monoclonal antibody isotype switch variants to *Cryptococcus neoformans* capsular polysaccharide were used to study the sensitivity of a double sandwich enzyme-linked immunosorbent assay (ELISA). The most sensitive ELISA configurations used IgG1 monoclonal antibody absorbed on polystyrene plates or IgM immobilized with goat antisera for antigen capture.

Cryptococcus neoformans infections are usually accompanied by the presence of capsular polysaccharide in body tissues (1, 10). The main component of *C. neoformans* capsular polysaccharide is glucuronoxylomannan (GXM) (6). Detection of cap-

sular polysaccharide is useful for the diagnosis of cryptococcal infection (1, 10). Currently, most clinical laboratories use latex agglutination assays. However, occasional reports of negative latex agglutination assays for fluids positive for *C. neoformans*

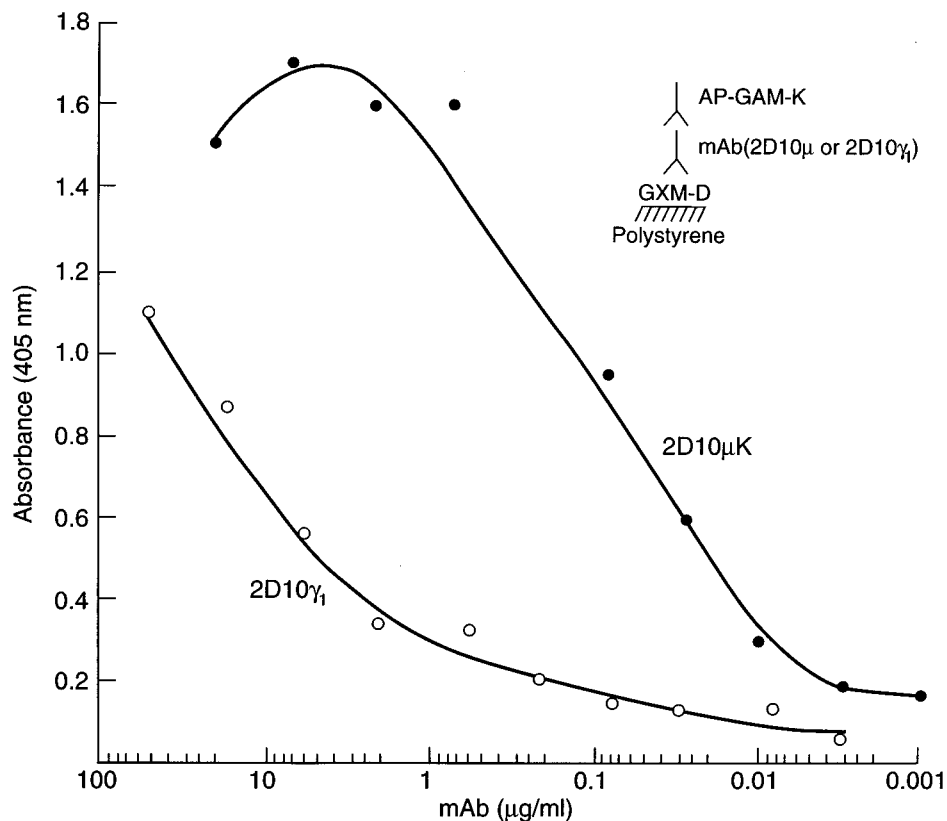


FIG. 1. Binding of MAbs 2D10 μ and 2D10 γ 1 to GXM adsorbed on polystyrene. The ELISA was done as previously described (2). Plates were made by incubating 50 μ l of 2- μ g/ml GXM in PBS in each well overnight as previously described (3). Points are the averages of four measurements. The ELISA configuration is diagrammed. AP-GAM- κ , alkaline phosphatase-labelled GAM κ -specific antisera.

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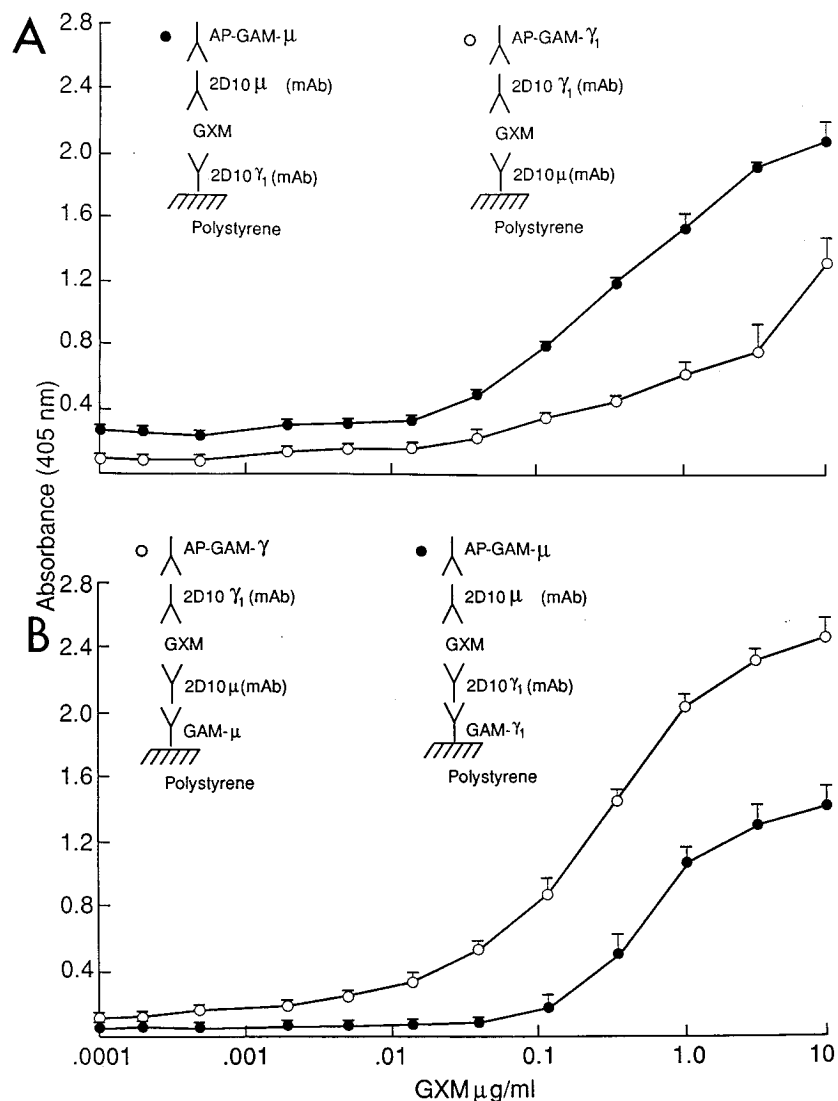


FIG. 2. GXM detection by MAb sandwich ELISAs. (A) Binding curves from capture ELISAs in which polystyrene MAbs were used to capture and immobilize GXM. Capture antibody was incubated with polystyrene plates at a concentration of 2 $\mu\text{g/ml}$ in PBS. (B) Binding curves from capture ELISAs in which the capture antibodies were themselves captured with GAM isotype-specific sera (0.2 $\mu\text{g/ml}$). In both types of ELISAs, GXM was detected by a 2D10 MAb of different isotype followed by the addition of alkaline phosphatase (AP)-labelled GAM isotype-specific antibody and *p*-nitrophenyl phosphate substrate. Points are the averages of four measurements, and brackets denote standard deviations. The MAb sandwich configuration for each type of assay is diagrammed.

by culture suggest the need for improved antigen detection assays (7). Several enzyme-linked immunosorbent assays (ELISAs) for the detection of cryptococcal antigen, some of which are more sensitive than latex agglutination assays, have been described elsewhere (2, 14, 16). Polysaccharides for some *C. neoformans* strains bind poorly to polystyrene plates; this has resulted in the use of polylysine (15), protein adipic acid dihydrazide derivatives (4), and antibody capture to immobilize polysaccharide (2, 14, 16). Recently, a detection system which is based on a two-site noncompetitive ELISA and utilizes polyclonal sera for antigen capture and a GXM binding monoclonal antibody (MAb) for antigen detection (9) has been introduced into clinical practice.

To study the contribution of isotype (and avidity) to the sensitivities of sandwich ELISAs, we used an immunoglobulin G1 (IgG1) isotype switch variant of murine IgM MAb 2D10 (13). IgM MAb 2D10 (2D10 μ) was isolated from a BALB/c mouse immunized with a GXM-tetanus toxoid vaccine and is

remarkable for having the highest apparent affinity in our MAb set (11). IgG1 MAb 2D10 (2D10 γ_1) is an isotype switch variant of MAb 2D10 which was isolated by sib selection as modified by the ELISA spot technique (13). MAbs 2D10 μ and 2D10 γ_1 have the same specificity but differ in isotype (13).

Ascites fluid containing MAb was obtained by paracentesis from BALB/c mice with hybridoma cells injected into the peritoneal cavity. MAb 2D10 γ_1 was purified by protein G affinity chromatography (Pierce, Rockfield, Ill.). MAb 2D10 μ was purified by mannan affinity chromatography (Pierce). Cetyltrimethylammonium bromide precipitation was used to isolate GXM from culture supernatant of strain ATCC 24067 (American Type Culture Collection, Rockville, Md.) (5). GXM from strain 24067 binds well to polystyrene plates (2). GXM ELISAs used MAbs of different isotypes to capture and detect GXM and were performed as previously described (2, 3). For GXM capture, isotype variants were either absorbed directly on polystyrene or themselves captured with goat anti-mouse immuno-

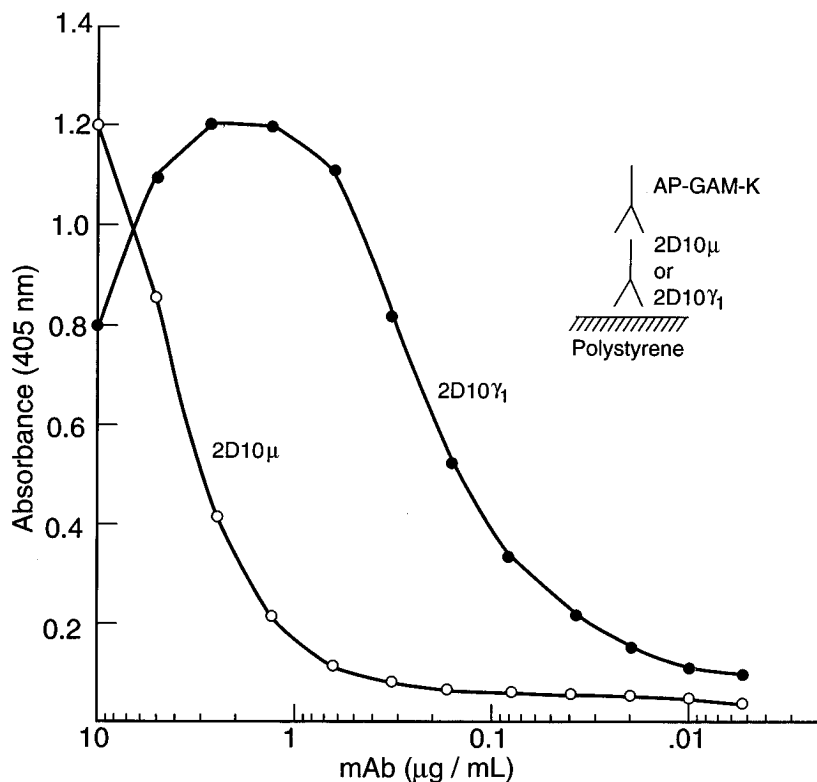


FIG. 3. Absorption of MAbs 2D10 μ and 2D10 γ 1 to polystyrene. The binding of MAbs 2D10 μ and 2D10 γ 1 was detected by absorbance after incubation with GAM κ -specific antisera (0.2 μ g/ml) followed by incubation with *p*-nitrophenyl phosphate substrate. The MAb sandwich configuration for each type of assay is diagrammed. Points are the averages of four measurements. AP, alkaline phosphatase.

globulin (GAM) isotype-specific sera (Fisher Scientific, Orangeburg, N.Y.) (2). All assays were done on Corning polystyrene ELISA plates (Corning Glass Works, Corning, N.Y.) coated with antibody solutions in 0.02 M phosphate-buffered saline (PBS), pH 7.2. Blocking for nonspecific binding was done with 1% bovine serum albumin in PBS. Incubations were for at least 1.5 h at 37°C. Mouse MAb detection was done with alkaline phosphatase-labelled GAM isotype-specific reagents (Fisher Scientific). A_{405} was measured in a Ceres 900 reader (Bio-Tek Instruments, Inc., Winooski, Vt.).

MAb 2D10 γ 1 has a lower affinity for polystyrene-bound GXM than does 2D10 μ (Fig. 1), presumably as a result of a loss of avidity on switching from a decavalent IgM to a divalent IgG. This is a common finding when IgM MAbs are switched to IgG isotypes, and similar results have been noted for mouse-human IgG1 chimeric antibody constructs of MAb 2D10 μ (17). To determine the most sensitive MAb 2D10 isotype configuration, IgM and IgG1 isotype variants were studied in capture and detection modes. When MAbs 2D10 μ and 2D10 γ 1 were added directly to polystyrene plates for GXM capture, IgG1 was more sensitive than IgM (Fig. 2A). The finding that capture by polystyrene-absorbed 2D10 μ was less sensitive than capture by polystyrene-absorbed 2D10 γ 1 was surprising, given the higher avidity of 2D10 μ . To investigate whether differences in isotype binding to polystyrene were responsible for the decreased sensitivity of IgM, plates were incubated with various concentrations of 2D10 μ and 2D10 γ 1, and MAb bound to polystyrene was detected with alkaline phosphatase-labelled κ -specific GAM (Fig. 3). Binding curves show that less 2D10 μ than 2D10 γ 1 was bound to polystyrene. The decreased binding

of the IgM isotype to polystyrene provides an explanation for the lower sensitivity of 2D10 μ in the capture mode. However, in ELISAs in which the capture MAb was itself captured by GAM isotype-specific sera, the combination of capture with 2D10 μ and detection with 2D10 γ 1 was more sensitive for the detection of GXM (Fig. 2B).

MAbs 2D10 μ and 2D10 γ 1 can modify the course of *C. neoformans* infection in mice (12, 13). Since 2D10 μ and 2D10 γ 1 have the same specificity, the finding that this pair can be used for capture and detection of GXM implies that GXM has at least two epitopes that are accessible to this MAb specificity. The occurrence of multiple identical epitopes in GXM is not surprising, since its structure has been proposed to be a repeating substituted mannose subunit (6). Nevertheless, this observation shows that GXM molecules are polyvalent for at least one epitope.

In summary, the sensitivities of GXM capture ELISAs with IgM and IgG1 MAb switch variants are dependent on the isotype configuration of the antibody sandwich. For 2D10 γ 1, absorption in polystyrene resulted in a more sensitive ELISA than capture by GAM-IgG1. For 2D10 μ , capture by GAM-IgM resulted in a more sensitive ELISA than absorption to polystyrene. Differences in isotype binding to polystyrene probably account for the differences in ELISA sensitivity. The type of ELISA described here has been shown to be useful for the measurement of GXM in mice infected with *C. neoformans* (13) and in serum samples from patients with cryptococcosis (8). Isotype configuration appears to be an important consideration in the design of antibody capture ELISAs for *C. neoformans* GXM.

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