Galactoxylomannan Does Not Exhibit Cross-Reactivity in the Platelia Aspergillus Enzyme Immunoassay

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Given the recent report of a false-positive result in the Platelia Aspergillus enzyme immunoassay in a patient with cryptococcosis and in yeast extracts and purified galactoxylomannan of Cryptococcus neoformans, we evaluated culture extracts, purified polysaccharides, clinical specimens, and specimens from animals following experimental infection. Our results revealed no cross-reactions.

Galactomannans (GalMs) are complex polysaccharides composed of d-galactose and d-mannose. Alkaline-extracted GalMs from Aspergillus fumigatus contain a core of (1→6)-α-d-mannopyranosyl residues, with β(1→5)-linked β-galactofuranosyl units. The pathogenic fungus Cryptococcus neoformans also contains complex capsular polysaccharides composed of glucuronoxylomannan (GXM) and galactoxylomannans (GalXM). GXM makes up about 90% of the capsular mass and is a polymer of up to six different repeating units that consist mostly of a linear α(1→3)-mannan trisaccharide with side groups consisting of β(1→2)-glucopyranosyluronic acid and β(1→2)- and β(1→4)-xylopyranosyl (2, 6). Additionally, the mannan backbone of GXM is modified by acetyl groups. GalXM constitutes about 7% of the capsular mass (2) and has an α(1→6) galactan backbone containing four potential short oligosaccharide branch structures. The branches are 3-O-linked to the backbone and consist of an α(1→3)-Man, α(1→4)-Man, β-galactosidase trisaccharide with variable amounts of β(1→2)- or β(1→3)-xylose side groups (Fig. 1) (2). The GalXM backbone consists of galactopyranose and a small amount of galactofuranose (11), unlike GXM, which contains only mannopyranose (2). A recent study showed that GalXM was significantly smaller than GXM (1.7 × 10⁶ Da), with an average mass of 1 × 10⁶ Da (6). Since GalXM has a smaller molecular mass, this implies that GalXM is the most numerous polysaccharide in the capsule on a molar basis, since there are 2 to 3.5 mol of GalXM for each mole of GXM. However, the often assumed conclusion that GXM was the most abundant molecule was based on the total amount of material that was recovered from the culture supernatant. These capsular components are shed into culture media, and GXM is found in body fluids of patients, assisting in the diagnosis of cryptococcosis. However, whether GalXM is present in body fluids is unknown, as there is no diagnostic test available for its detection.

The cell wall GalM of Aspergillus spp. is likewise shed into culture media and body fluids of patients with aspergillosis, and it is the target of the Platelia Aspergillus enzyme immunoassay (EIA). β(1→5)-Galactofuranosyl side chains are the antigenic sites recognized by the monoclonal antibody used in the Platelia Aspergillus EIA (8).

Recently, the Platelia Aspergillus EIA was noted to be positive in a patient with cryptococcal meningitis (4). In that case, however, the patient was also receiving amoxicillin-clavulanate, a drug associated with false-positive results in the Platelia Aspergillus EIA. GalXM at concentrations of 100 ng/ml or higher also gave positive results in the Platelia Aspergillus EIA. GXM, however, the target of the cryptococcal latex agglutination (LA) test, was negative in the Platelia Aspergillus EIA. The investigators also demonstrated that C. neoformans yeast suspensions were positive in the Platelia Aspergillus EIA. Cross-reactivity with C. neoformans was not observed in earlier studies of the Platelia Aspergillus EIA (9) or the monoclonal antibodies used in that assay (8). In view of these conflicting findings, we undertook additional studies of cross-reactivity with C. neoformans in the Platelia Aspergillus EIA.

Cryptococcal GXM was prepared from C. neoformans strain 24067 and GalXM and GalXM mannoprotein from strain cap67 according to published methods (3, 10, 11). Compositional analysis of GalXM was confirmed by gas chromatography/mass spectrometry of the per-O-trimethylsilyl derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis. The analysis revealed the moles percentages for GalXM’s components were as follows: xylose, 17%; mannose, 28%; and galactose, 55% (10). These numbers are very similar to the moles percentages in Cherniak’s studies: xylose, 22%; mannose, 29%; and galactose, 50% (11). Addi-
FIG. 1. Comparison of the structures of the major repeating units of GalM from *Aspergillus* spp. (13) and GalXM and GXM from *C. neoformans* (2). The lack of antigenic cross-reactivity between GalM and GalXM in the EIA is apparent in the comparison of these structures. In comparison to the β(1→3)-galactofuranose side chain linked to an α(1→2)-mannotetraose core by an α(1→6) bond in GalM, GalXM differs by the absence of β(1→5)-galactofuranose side chains and the presence instead of β(1→3)-xylomannan side chains and an α(1→6)-galactopyranose core. The lack of structural similarity between the GalM and GXM further reflects the lack of cross-reactivity of the two antigens in the anti-GalM EIA. Green, galactofuranose or galactopyranose; blue, mannopyranose; red, xylose; gray, glucuronic acid. The asterisk near the 1→3 galactofuranosylmannopyranose bond indicates that there is currently uncertainty as to whether this linkage is α or β in configuration.
tionally, we ran a proton nuclear magnetic resonance spectrum for GalXM without further purification through an ion-exchange column as Cherniak had done earlier (11). The analysis showed that the anomeric region was between 5.4 and 4.3 ppm. The results were compared semiquantitatively on a scale from 0 to 5+, with results from 1+ to 5+ considered to be positive. Cn, C. neoformans.

The cryptococcal LA titer ranged from negative (one sample) to 1:65,336 (median, 1:1,024), but all were negative in the Platelia Aspergillus EIA. We also tested residual serum specimens from 20 patients that were positive in the Platelia Aspergillus EIA. The Aspergillus GalM GalM galactomannan index units (GMI) ranged from 0.5 to 10.4 (median, 1.9), but all were negative in the cryptococcal LA test (Table 2).

Cross-reactivity was also studied in experimental models of invasive aspergillosis and cryptococcosis, according to institutional guidelines. BALB/c 6- to 8-week-old female mice (n = 8) from the National Cancer Institute (NCI) were infected intravenously with 1 × 10⁴ organisms of C. neoformans strain 24067 (1). The mice were sacrificed 7 days postinfection by asphyxiation with CO₂, and sera and lungs were immediately harvested, placed in ice-cold phosphate-buffered saline, and frozen at −70°C. Tissues were placed in 2 ml of F-12 nutrient medium (Invitrogen Corporation, Carlsbad, CA) supplemented with glucose, glutamic acid, cystine, and HEPES and homogenized in sterile Ten Broeke (Vineland, NJ) grinders. The undiluted homogenates were positive in the cryptococcal LA test, with agglutination scores of 3+ or 4+ for five, 2+ for two, and 1+ for one animal. All were negative in the Platelia Aspergillus EIA.

Pulmonary aspergillosis in New Zealand White rabbits (n = 7) (Hazelton Research Products, Inc., Denver, PA) was established by inoculating them intratracheally with A. fumigatus (NIH isolate 4215; ATCC no. MYA-1163) (7). The studies were approved by the Animal Care and Use Committee of the NCI, and the rabbits were housed and monitored in facilities accredited by AAALAC International according to guidelines of the Public Health Service Policy for the Care and Use of Laboratory Animals. The rabbits were euthanized by intravenous administration of pentobarbital (65 mg/kg of body weight of pentobarbital sodium in the form of 0.5 ml of beuthanasia-D special euthanasia solution; Schering-Plough Animal Health Corp., Union, NJ). Undiluted plasma and bronchoalveolar lavage (BAL) fluid were positive in the Platelia Aspergillus EIA: the GMI ranged from 0.8 to 4.2 (median, 2.4) in plasma and 1.5 to 6.8 (median, 6.4) in BAL fluid. All specimens were negative in the cryptococcal LA test.

Cross-reactions between GXM, GalXM, and Aspergillus n

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GalM were not observed in this study. The reasons for the discrepant findings, compared to those of Dalle et al. (4), are unknown. The GalXM used in that study was prepared some years ago (5, 10) and may have changed during storage. Since the positive result in the Platelia Aspergillus EIA was noted only at high concentrations of cryptococcal GalXM (100 ng/ml), low-level contamination with Aspergillus GalM is also possible. The limit of detection of the Platelia Aspergillus EIA for Aspergillus GalM is about 0.5 ng/ml, permitting detection of low-level contamination. Also, subtle differences in methods for preparation of the GalXM may have contributed to the discrepant findings. Nevertheless, the biochemical and nuclear magnetic resonance characteristics of our GalXM were similar to that prepared by Cherniak et al. (5, 10), which was used in Dalle’s study (4).

Previous studies had failed to observe cross-reactivity in culture supernatants from C. neoformans in the Platelia Aspergillus EIA (8, 9). Furthermore, positive results in the Pastorex Aspergillus LA test were not detected in plasma from guinea pigs with cryptococcosis (12).

A biochemical basis for cross-reactivity between cryptococcal GalXM and Aspergillus GalM is not obvious (2, 13). The structural dissimilarities among GalM, GalXM, and GXM suggest that the likelihood for shared antigenicity may be small (Fig. 1). Although β-galactofuranose, the epitope recognized by the monoclonal antibody used in the Aspergillus GalM antigen assay (8), is present in small amounts in GalXM (11), it is unknown if the two are similar antigenically. Thus, we were unable to confirm the cross-reactivity of C. neoformans GalXM or culture extracts or false-positive results in specimens from patients or animals with cryptococcosis in the Platelia Aspergillus EIA.

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