VARIABILITY IN GALACTOMANNAN DETECTION BY PLATELIA Aspergillus EIA™ ACCORDING TO THE Aspergillus SPECIES

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SUMMARY

Here we investigate the extent to which different Aspergillus species release galactomannan (GM) in vitro. Marked variability was observed in GM reactivity between and within Aspergillus species, with A. terreus strains showing the highest GM indexes. The in vivo significance of these findings remains to be determined.

KEYWORDS: Invasive aspergillosis; Galactomannan; Diagnosis.

INTRODUCTION

Galactomannan (GM) is a thermosstable polysaccharide that is a component of the cell wall of a diverse range of fungi, including those belonging to the genus Aspergillus. Since GM is released during fungal hyphal growth, its detection in circulation or in other body fluids allows for an early diagnosis of invasive aspergillosis (IA). However, when interpreting a positive GM result in a patient with suspected IA, one might consider the variety of factors that can potentially interfere with the test, including antibiotic use, dietary factors and cross-reactivity with non-Aspergillus fungi. Although early investigations have suggested that GM levels may vary among Aspergillus species, there is still only very limited data to support this. Here we report that marked inter- and intra-species variation occurs in the GM release in vitro.

MATERIALS AND METHODS

Exoantigen testing was based on the method described by SWANKINK et al., in 12 well-characterized Aspergillus strains. All strains were kindly provided by Myconostica (National Aspergillosis Centre, UK). These included A. fumigatus (n = 4) strains AF294, AF10, AF71, and AF13073; A. flavus (n = 2), AF5, and AF16883; A. niger (n = 3), An9029, An1015, and An186; A. terreus (n = 2), At10071, and At49; and A. nidulans (n = 1), strain NEQAS UK. In brief, strains were subcultured to obtain pure young cultures in Sabouraud at 25 °C for 48 h (A. fumigatus, A. flavus and A. niger) or 96 h (A. terreus and A. nidulans). One loop of each strain was used to prepare the inoculum in liquid Sabouraud medium, which was adjusted by spectrophotometry (530 nm) to 80-82% T (2x10^6 to 2.5x10^7 CF/mL). Strains were incubated at 35 °C for 48-96 h, centrifuged for five min with 2500 rpm and then filtered (Millipore 0.45 µm). Tenfold dilutions were applied successively, and reactivity of the sandwich ELISA to GM detection was determined in duplicate at the 10^-6 dilution, using Platelia Aspergillus EIA kit (BioRad). The Platelia Aspergillus test was performed according to manufacturer’s instructions. In short, 100 µL of Platelia treatment solution (4% ethylenediaminetetraacetic acid solution) was added to 300 µL of the adjusted innoculum, homogenized, and heated to 120 °C for six min in a heat block, followed by centrifugation at 10,000g for 10 min. Next, 50 µL of the supernatant and 50 µL of the horseradish peroxidase labeled monoclonal antibody (EBA-2) were incubated in antibody precoated microplates for 30 min in the dark at room temperature. The reaction was stopped with 1.5 N sulfuric acid solution, and the plates were read at an optical density (OD) of 450 nm, with a reference filter of 620/630 nm. Positive, negative, and cut-off controls were incorporated in each assay. GM results were expressed as optical densities (OD) – samples were considered positive when the ratio between the OD observed for the sample and the mean cut-off OD was > 0.5. Strains resulting in negative GM readings were again tested in duplicate at the previous dilution (10^-6).

RESULTS

Median GM indexes (range) at the 10^-6 dilution were: A. terreus, 3.82 (1.30 to > 6.35); A. nidulans, 1.3; A. fumigatus, 0.88 (0.17-1.50); A. niger, 0.28 (0.24-0.79), and A. flavus, 0.16 (0.14-0.18). Considering A. fumigatus as the comparator, GM reactivity for the other Aspergillus species was 434% for A. terreus, 148% for A. nidulans, 32% for A. niger, and 18% for A. flavus. At the 10^-4 dilution 50% of the A. fumigatus strains...
Table 1
Marked variations are observed in galactomannan reactivity when different Aspergillus species and strains are tested in vitro. All experiments were performed in duplicate at the 10<sup>-6</sup> dilution using Platelia Aspergillus EIA kit (Bio-Rad). Optical densities represent mean values

<table>
<thead>
<tr>
<th>Aspergillus strain</th>
<th>Galactomannan optical density</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. fumigatus (AF 294)</td>
<td>1.50</td>
</tr>
<tr>
<td>A. fumigatus (AF 10)</td>
<td>0.33</td>
</tr>
<tr>
<td>A. fumigatus (AF 71)</td>
<td>0.17</td>
</tr>
<tr>
<td>A. fumigatus (AF 13073)</td>
<td>1.43</td>
</tr>
<tr>
<td>A. flavus (Af 5)</td>
<td>0.18</td>
</tr>
<tr>
<td>A. flavus (Af 16883)</td>
<td>0.14</td>
</tr>
<tr>
<td>A. niger (An 9029)</td>
<td>0.28</td>
</tr>
<tr>
<td>A. niger (An 1015)</td>
<td>0.79</td>
</tr>
<tr>
<td>A. niger (An 186)</td>
<td>0.24</td>
</tr>
<tr>
<td>A. terreus (At 10071)</td>
<td>1.30</td>
</tr>
<tr>
<td>A. terreus (At 49)</td>
<td>&gt; 6.35</td>
</tr>
<tr>
<td>A. nidulans (NEQAS UK)</td>
<td>1.30</td>
</tr>
</tbody>
</table>

(AF10 and AF71) were GM negative, as well as the two A. flavus strains and two out of the three A. niger strains tested (An186 and An9029). These were all positive at 10<sup>-3</sup> dilution, with GM indexes varying from 0.51 to 1.02.

DISCUSSION

This study showed that marked variations occurred in GM release among Aspergillus species, with the highest GM indexes being observed for A. terreus. On the other hand, A. niger and A. flavus showed less reactivity in the GM ELISA test, in comparison to A. fumigatus. Previous studies had already suggested that in vitro GM release may vary according to the Aspergillus species being tested<sup>16</sup>. However, varied results have been documented in our study as well as among different strains belonging to the same species. It remains to be determined whether such differences could affect the determination of GM concentrations in vivo.

If the results of our study are confirmed in vivo, then differences in serum GM levels observed among patients with IA may also be related to the Aspergillus species causing the infection. As a result, caution would be required when diagnosing IA based on the use of a universal cut-off for serum GM detection (i.e., 0.5). In fact, a clinical study involving haematological patients showed higher sensitivity of the Platelia Aspergillus EIA test in the diagnosis of IA when the infection was caused by Aspergillus species other than A. fumigatus (49% vs. 13%, in comparison to A. fumigatus, respectively)<sup>3</sup>. One interesting finding of our study was the observation that variability in GM levels also occurred within each of the Aspergillus species evaluated. For instance, the magnitude of such differences was as high as ninefold, and this could potentially explain the difference in results obtained among studies.

In conclusion, this study showed that marked variations occurred in GM levels among distinct Aspergillus species, as well as among different strains belonging to the same species. It remains to be determined whether such differences could affect the determination of GM concentrations in vivo.

RESUMO

Variabilidade na detecção de galactomanaana pelo Platelia Aspergillus EIA® de acordo com a espécie de Aspergillus


REFERENCES


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