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Reinvestigation of carbohydrate specificity of EB-A2 monoclonal antibody used in the immune detection of *Aspergillus fumigatus* galactomannan

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Abstract

Great progresses have been made in the recent years in the detection of circulating galactofuranose-bearing molecules for the diagnosis of aspergillosis. However, the test used in the clinical practice is hampered by the occurrence of false positives. A glycoarray with dozens of oligosaccharides structurally related to the *Aspergillus fumigatus* galactomannan has allowed us to reinvestigate the carbohydrate specificity of the EB-A2 monoclonal antibody used in the Platelia™ Aspergillus sandwich immune assay. We have now demonstrated that the mAb can recognize shorter oligosaccharides than the previously reported tetrasaccharide Gal*f*-β-(1→5)-Gal*f*-β-(1→5)-Gal*f*-β-(1→5)-Gal*f*-β and oligosaccharides which contains alternating β-(1→5)/β-(1→6)-linkages. This result could explain the occurrence of false-positive signals due to the presence of the abovementioned

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epitopes not only in *A. fumigatus* galactomannan but also in other bacteria and fungi.

Keywords: Health sciences, Biochemistry, Immunology, Microbiology, Infectious disease

1. Introduction

The detection of circulating galactomannan (GM) is a recognized criterion for the diagnosis of invasive aspergillosis [1, 2, 3]. The commercial kit Platelia™ Aspergillus enzyme immunoassay used in the clinics is based on an immunoassay with the rat IgM EB-A2 which has been shown to recognize oligosaccharides composed of at least four β-(1→5)-galactofuranosyl units [4, 5]. This epitope is present on the galactomannan, which is a major component of the Aspergillus cell wall and occurs as side chains of the linear mannan core. Despite a good sensitivity and specificity, the performance of the Platelia™ Aspergillus GM immunoassay has been shown to be far from perfect, particularly among patients receiving mold-active prophylaxis or treatment or pediatric patients [6, 7]. This is mainly due to the occurrence of false positives encountered for this test.

Many reasons have been proposed to be responsible for false positive results with the commercial GM test. First, some of the false positive results come from an erroneous operational use of the test [8]. Some other false positive data result from the presence of GM in the circulating fluid of the patient which was not linked to an *Aspergillus* infection: the major example is connected with the use of Tazocin™ (Piperacilin-Tazobacam) whose raw material utilized for the production of the antibiotics was contaminated by filamentous ascomycetes such as *Penicillium* or *Aspergillus* which both are source of GM. The use of Tazocin™ which is not contaminated by GM solved this problem [9].

False positive data have been also associated with intake of contaminated food and enteral nutrition [10]. Similarly to Tazocin™, a better purification of solutions containing low-molecular-weight organic acids which are produced by a fermentation process involving *Aspergillus* resolved these problems like in cases of false positivity due to the intravenous injection of sodium gluconate [11]. Other invasive fungal infections are suspected to be associated with the release of GM: (i) it has been demonstrated chemically that *Penicillium* secretes a polysaccharide with a β-(1→5)-galactofuranoside epitope similar to the one present in *Aspergilli* species [12] and that the GM test can be used to detect *Penicillium marneffei* infections (Huang et al, 2007) [13]; (ii) a positivity of the ELISA test has been noticed with the yeasts *Cryptococcus* and *Histoplasma* while the presence of β-(1→5)-galactofuranoside units has not been reported in these yeast species. Some of the false

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positives still remain unexplained such as the positivity of multiple myeloma patients exempt of aspergillosis infections [14].

The false positivity can also result from cross reactions knotted with the presence of different bacteria of the human microbiota including *Escherichia coli* [15], *Rhodococcus equi* [16], *Corynebacterium jeikeium* [15] and *Bifidobacterium* spp [17]. They produce a galctofuranoside epitope also recognized by the EB-A2 mAb and may be responsible for false positivity in patients in the late phase of allogeneic hematopoietic stem cell transplantation with heavy gastrointestinal Graft vs Host disease [18]. Moreover, (i) N-glycans and glycolipids which do not bear the tetragalactofuranosyl moiety are recognized by this monoclonal antibody [19] and (ii) recent studies have shown that side chains of GM are not exclusively composed of linear β-(1→5)-galactofuranosyl units [20] but contain a certain amount of β-(1→6)-linked galactofuranosyl units attached to the mannan backbone [21, 22]. All these data have raised some questions on the exact nature of the epitope recognized by the EB-A2 mAb and suggested that the mAb used in the commercial kit may recognize multiple carbohydrate epitopes, a classical fact with anti-carbohydrate antibodies. The multiplicity of the epitopes recognized may be also a reason for the occurrence of some of the false positives reported in the literature which affects the performance of the test for the diagnosis of invasive aspergillosis.

2. Results & discussion

To reinvestigate the nature of the carbohydrate epitope recognized by the mAb EB-A2 a glycoarray composed of synthetic oligosaccharides with definite structures representing key fragments of the galactomannan of *A. fumigatus* was used. The selection of synthetic oligosaccharide derivatives **1**−**13** (Fig. 1A) for this study was based on the most recent definition of the galactofuranyl-containing structures of *Aspergillus* galactomannan. Oligosaccharides **1**−**13** were prepared [22, 23, 24] using pyranoside-*into*-furanoside rearrangement [25, 26] and biotynilation using an activated biotin derivative [27] containing a hydrophilic hexaethylene glycol linker. The wells of 96-well streptavidin-coated plates (Thermo Scientific, Rockford, U.S.A.) were coated with biotin-tagged oligosaccharides at a concentration of 15 pmol/well. After two hours incubation with EB-A2 conjugated with peroxidase (working solution from the Platelia *Aspergillus* Ag Kit) and revealed following the instructions of the manufacturer.

The use of the glycoarray has expanded the number of oligosaccharide ligands recognized by mAb EB-A2. The minimal recognized galactomannan fragment is a disaccharide Gal*f*-β-(1→5)-Gal*f*. Moreover, this disaccharide fragment is recognized as the terminal part of the polysaccharide chain (as in compounds **2**, **6**, **8**, **9**, **10**, **11**−**13**) or within the internal part of the chain (see compound **7**). In addition,

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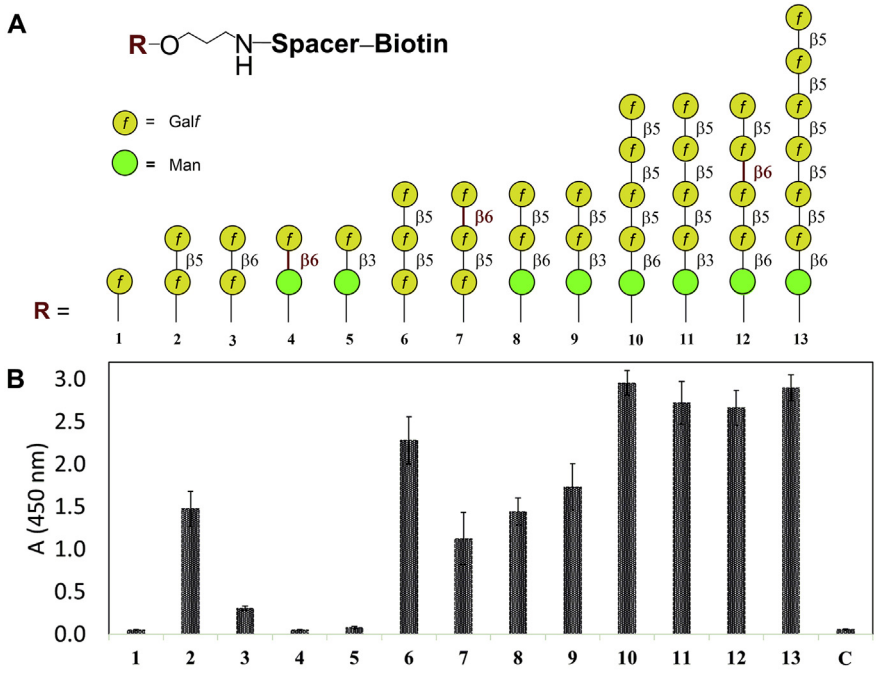

Fig. 1. Investigation of the oligosaccharide specificity of EB-A2 mAb. (A) The thematic glycoarray composed of oligosaccharide ligands representing key structural elements of the *A. fumigatus* galactomannan chain, and (B) the results of assaying the carbohydrate specificity of EB-A2 mAb on the glycoarray.

the data shown in Fig. 1B confirmed the results of earlier studies [5], demonstrating that the oligosaccharide ligand with four Gal*f*-β-(1→5) units belongs to the best recognized sequences. However, no difference was observed between the isomeric pentasaccharides **10** and **12,** which contained only β-(1→5) linkages or alternating β-(1→5) and β-(1→6) linkages in their tetragalactofuranosyl fragments. This observation may explain why the structurally different polysaccharides of *B. bifidum* and *B. catenulatum* are recognized by EB-A2 mAb [28, 29], since *B. bifidum* produces lipoteichoic acid polysaccharide containing an oligo-β-(1→5)-galactofuranosyl backbone [30], while *B. catenulatum* produces a polysaccharide with alternating β-(1→5)- and β-(1→6)-galactofuranosyl units [31].

3. Conclusion

This study indicates that the mAb EB-A2 used in the kit for the detection of the circulating GM for the diagnosis of aspergillosis, recognizes multiple epitopes that are all present in the native GM molecule. The multiplicity of the epitopes recognized by the mAb can be a major cause for the occurrence of false positive results which impacts the performance of the existing test. Substitution of EB-A2 mAb in the immune assay with an antibody capable of recognizing a larger epitope should increase the specificity of the assay and will facilitate the decision for the initiation of

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an antifungal therapy. Synthetic immunogens which contain the oligosaccharide ligands of necessary length and structure can be regarded as promising instruments for obtaining of monoclonal antibodies with required epitope specificity and affinity to GM [32, 33] which may be better adapted for the conception of a more specific test in the serological diagnosis of invasive aspergillosis.

Declarations

Author contribution statement

Vadim B. Krylov, Arsenii S. Solovev, Dmitry A. Argunov: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Jean-Paul Latgé, Nikolay E. Nifantiev: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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