

Cross-Reactivity of Anti-Galactomannan Antibody EB-A2 with Lipoteichoic Acid of *Bifidobacterium bifidum* subsp. *pennsylvanicum*

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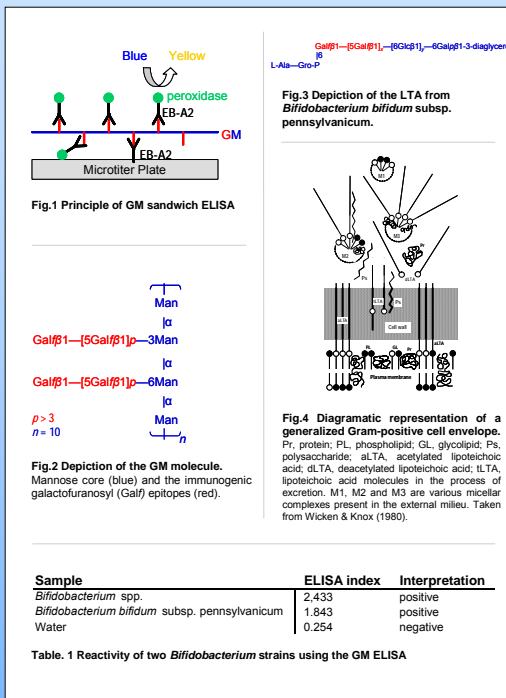
1. Introduction

Prospective studies have shown that circulating galactomannan (GM) can be detected with high sensitivity and specificity and at an early stage of infection in patients with invasive aspergillosis (IA), especially when serial serum samples are tested^{6,7}. GM is a fungal cell wall polysaccharide released by *Aspergillus* species during growth^{13,14}. GM can be detected by the commercially available sandwich ELISA (Platelia® *Aspergillus*, BioRad, France) where the rat monoclonal antibody EB-A2 is used as captor and detector (Fig.1). This test has a detection limit of 1 ng/ml GM in serum^{13,14}. Although GM is an early and specific marker of IA in adults, false-positive ELISA reactivity is found in up to 83% of neonates^{11,12,15}. In neonates persistent ELISA reactivity is found in consecutive samples^{11,12}. One explanation is the gastrointestinal translocation of fungal GM from contaminated food in patients with an immature mucosa or a reduced integrity of the intestinal mucosa barrier due to cytotoxic chemotherapy¹. However, antigenemia is expected to be variable when related to cross-reacting components in the patients' diet while especially neonates were found to have persistent antigenemia. An alternative explanation is cross reactivity of the rat EB-A2 monoclonal IgM antibody with other molecules present in serum.

Aim: The literature was systematically reviewed for cross-reacting epitopes that could be recognized by the EB-A2 monoclonal antibody.

2. Methods & Results

The epitope recognized by EB-A2 is the $\beta(1-5)$ -linked galactofuranosyl chain of the GM molecule of which at least 4 residues should be present to allow binding(Fig.2)⁴. Copernic Agent Professional 6.1 was used to conduct a search with 'galactofuranosyl' as the keyword. This search revealed articles describing galactofuranosyl as part of a lipoteichoic acid (LTA), a universal cell wall component of gram-positive bacteria(Fig.4)¹⁰. Fischer et al. and Op den Camp et al. describe the structure of lipoteichoic acid as a membrane-associated molecule of *Bifidobacterium bifidum* subsp. *pennsylvanicum*^{3,8}. This LTA contains a terminal linear polysaccharide of more than 7 $\beta(1-5)$ linked galactofuranosyl groups(Fig.3)³. *Bifidobacterium* spp. are common members of the gastrointestinal microflora of humans, comprising up to 3% of the total fecal microflora of adults and forming up to 91% and 75% of the total intestinal microflora in respectively breast-fed and milk-formula-fed infants⁹.



References

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2. Methods & Results (continued)

It is known that the propensity for transmucosal passage of bacteria or antigens in preterm neonates is increased and experimental studies have shown that translocation of *Bifidobacterium* species can occur due to a reduced integrity of the gastrointestinal mucosa during sepsis and upon exogenous administration of high doses of *Bifidobacteria*^{5,9}. The high load of *Bifidobacterium* species in the gut of neonates corresponds with the high number of neonates that show false-positive reactivity with serum. Furthermore, gastrointestinal colonization can act as a continuous source of LTA which is consistent with the observation of persistent serum ELISA reactivity in neonates. We hypothesized that the LTA of *Bifidobacterium bifidum* subsp. *pennsylvanicum* could cross-react with the EB-A2 monoclonal IgM antibody.

The *in vitro* reactivity in the GM sandwich ELISA (Platelia® *Aspergillus*, BioRad, France) was investigated by testing suspensions of *Bifidobacterium* spp. and *Bifidobacterium bifidum* subsp. *pennsylvanicum*. Bacteria were grown on yeast nitrogen base (Difco®) for 5 days. Afterwards the bacteria were suspended in water and used for testing in the ELISA according to the manufacturers' instructions. The medium as well as the water were also tested in the ELISA. According to the manufacturers' guidelines results are considered positive when the index defined as the optical density divided by the mean optical density of two threshold control sera is equal or larger than 1.5.

Cultures of both *Bifidobacterium* spp. and *Bifidobacterium bifidum* subsp. *pennsylvanicum* showed reactivity *in vitro* (Table 1).

3. Conclusions

The neonatal gut is heavily colonized with *Bifidobacterium* spp. and this bacterium or its LTA might cause ELISA reactivity with serum after translocation due to immaturity of the intestinal mucosa. Based on molecule similarity, we postulate that a lipoteichoic acid of *Bifidobacterium bifidum* subsp. *pennsylvanicum* can act as epitope for the monoclonal antibody used in the ELISA. Preliminary *in vitro* experiments confirm cross-reactivity, but further *in vivo* experiments are warranted.