X-ray structure of an anti-de-N-acetyl polysialic acid (dPSA) antibody complex and mechanism of MenB dPSA-mediated immune cell inhibition

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Abstract Information

Background

An antibody, SEAM 3, elicited by an N-propionyl MenB polysaccharide vaccine containing dPSA binds to MenB bacteria but does not cross-react with polysialic acid (polySia). In this study, we investigated the molecular basis for SEAM 3 dPSA-specificity and the interaction of MenB dPSA with immune cells to investigate the potential function of dPSA in MenB pathogenesis.

Aim/Methods

The SEAM 3 Fab/oligosaccharide complex was purified by SEC, and crystallized in 24% PEG 1,500/20% glycerol. X-ray diffraction data was collected at the Lawrence Berkeley Laboratory Advanced Light Source. The structure of the Fab was solved by molecular replacement and the bound sugar was built/refined with Privateer. Fluorescence microscopy and Amnis ImageStream was used to characterize dPSA transfer to immune cells. Siglec receptor specificity was determined by ELISA using recombinant siglec-Fc chimeras. To identify potential polySia de-N-acetylases, MenB dPSA was purified by detergent extraction and SEC. Proteins associated with MenB dPSA were identified by SDS-PAGE and LC-MS/MS mass spectroscopy.

Results

The structure of the Fab in complex with 4 Sia residues was obtained at 1.83Å resolution (free R factor of 0.229) with good geometry. The reducing end residue was in the open chain form, and one or two residues at the non-reducing end were de-N-acetylated. Most polar contacts with the Fab are with an N-propionylated second residue. The specificity for dPSA appears to be conformational and possibly involve charge-dipole interactions. dPSA and derivatives were found to bind to a specific siglec receptor with high affinity (KD=1nM). The siglec is expressed by monocytes, NK cells and a subset of T cells and is known to inhibit their activity. dPSA and lipoproteins from MenB were transferred from MenB to immune cells resulting in oligomerization of receptors. dPSA purified from MenB was 40% de-N-acetylated and two proteins, FrpC and NadA, were the only two proteins associated with it.

Conclusions

The specificity of SEAM 3 for dPSA depends on a confirmation which may be stabilized by inter/intra molecular charged interactions. MenB and human cancer cells that produce de-N-acetylated sialic acid-containing molecules may suppress the functional activity of immune cells by binding to a siglec receptor.