Overcoming the Glycan Blind Spot: Raising antibodies against immunorecessive carbohydrate-based antigens

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Abstract

Glycans are carbohydrate structures post-translationally conjugated to proteins and lipids which are typically well-tolerated by the immune system, as typified by limited self-glycan-reactive B cells present in the naïve repertoire. However, the resultant glycan immune 'blind spot' is readily exploited by cancers and viruses that acquire glycans to shield underlying sensitive surfaces from B cell recognition. We envisage that exploiting synthetic protein chemistry to install artificial 'near-self' glycans may selectively break glycan immune tolerance against a pathology-relevant target, balancing two diametrically opposed factors: 1) that such near-self glycans may be different enough from self to be recognized by the naïve B cell repertoire, but 2) similar enough to the desired target self-glycan that the antibodies raised are relevant, potentially requiring minimal somatic hypermutation to recognize the target glycan in its native context. To this end, we have developed a broadly translatable chemistry pipeline that efficiently installs carbohydrates onto lysine sidechains of model proteins, a conjugation chemistry absent from the mammalian glycome. Using classically immunosilent carbohydrates including the melanoma-associated 3-sialyllactose (SiaLac), the glycan component of ganglioside GM3, we demonstrate that this conjugation strategy raises isotype-switched antibodies in both wild-type and human immune system mice. Evaluation of the B cell clonality of these responses has uncovered glycan-engaging motifs in a narrow variable gene-segment subset consisting primarily of the phylogenetically related mouse IGHV2-3, IGHV2-6-5 and IGHV2-6-7 and IGHV2-9. Glycan-specific B cells largely outnumber carrier protein-specific B cells, suggesting efficient oligoclonal activation by SiaLac of a subset of highly related clones. Structural analyses reveal that accommodation of the unnatural lysine sidechain constrains recognition against the native, membrane-bound GM3 target, where specific light chain residues are predicted to sterically interfere with antibody binding. These findings may inform a novel germline-targeting approach to prime glycan-specific B cells relevant to cancer and pathogen carbohydrate antigens.