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# Detecting Glycosylation of Donor Specific Antibodies (DSA) with Single Antigen Beads

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## Short Communication

One of the major causes of graft loss among the kidney allograft recipients is the Antibody Mediated Rejection (AMR), and its manifestation depends on the circulating Donor Specific Antibodies (DSA) [1,2]. DSA can be detected quite reliably using Single Antigen Beads (SAB) technology with high sensitivity, but its clinical implication is not always straight forward or directly correlated with the incidence of AMR [3,4].

Immunoglobulins are glycoproteins, and it is well known that the glycosylation profiles of IgG dictate its functions [5,6], which is not well appreciated in the context of AMR yet. All human IgG has a common glycosylation site on the Fc region, and depending on the glycosylation status IgG may confer different properties (Figure 1). The G0 glycans have a higher affinity for Fc gamma receptor IIIa and mostly pro-inflammatory, while sialylated or focused states can be more anti-inflammatory since they have lower affinity to Fc gamma receptor IIIa. Also, for high affinity binding of C1q, the presence of galactose moiety is required [7]. Using these properties, glyco-engineering has been applied to IgG medications to develop more anti-inflammatory immunoglobulin based therapeutics [8]. Considering the impact of differential glycosylation of IgG on its effector functions, differential glycosylation of DSA will have corresponding impact on the manifestation of antibody mediated allograft injury.

Glycosylation profile of a protein can be studied in detail using various conventional methodologies, such as liquid chromatography and mass spectrometry [9]. Most of the glycosylation studies with this format were done in the context of overall serum IgG, but not to the specific level of anti-HLA antibodies. Requirement of purification of IgG may not be practical for hundreds of DSA analysis for different Human Leukocyte Antigens (HLA), and in this regard, use of single antigen beads have advantage in developing a practical assay to address glycosylation of DSA for clinical application.

For the application of glycan detection in the single antigen bead format, lectins have been used for specific and quantitative detection [10]. Specific recognitions of differential glycosylation states, such as G0, G1 or G2, sialylation and fucosylation, can be made with lectin based immunoassay (Figure 1). *Sambucus nigra* Agglutinin (SNA) recognizes sialylated glycans, and *Ricinus communis* Agglutinin (RCA) recognizes galactose residues while concanavalin A and *Aleuria aurania* lectin recognize mannose and fucose residues respectively. To be used with conventional flow cytometer, biotinylated lectins are used to be conjugated with neutravidin which has attached phycoerythrin reporter in our assay.

For the specific detection of glycans on DSA, other non-DSA glycoproteins need to be cleared for the assay, including single antigens on the beads. Class I HLA has a single conserved glycosylation site and class II HLA has three conserved glycosylation sites. Fully or partially de-glycosylated single antigen beads can be prepared with enzymatic glyco-engineering by using PNGase F for class I beads and neuraminidase for class II beads (data not shown).

Other than glycans on the single antigen beads, serum also has significant quantities of

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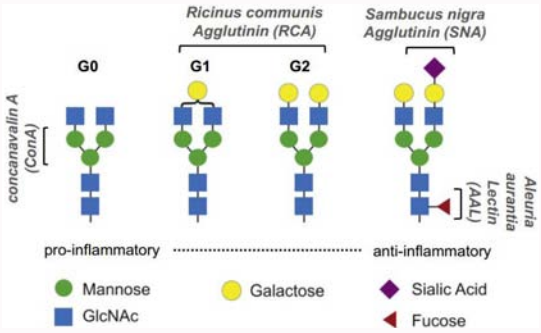


**Figure 1:** Glycosylation profile of IgG and specific Lectin probes (SNA, RCA, ConA and AAL).

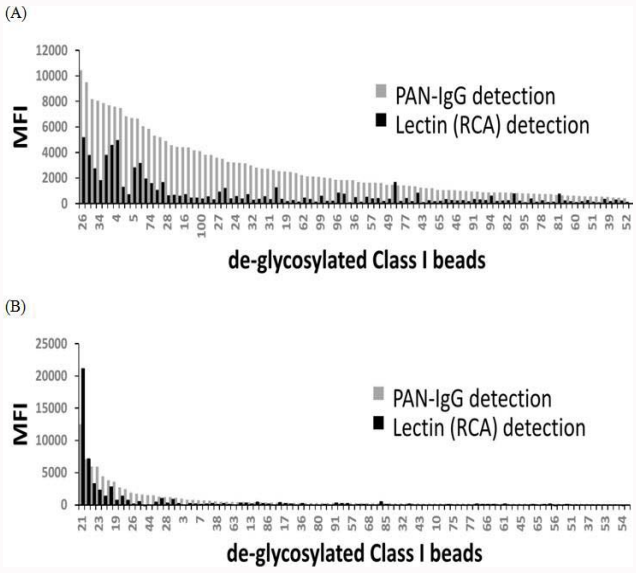


**Figure 2:** Differential glycosylation patterns of HLA antibodies from positive control sera (A) and a post-transplant patient's serum (B).

glycoproteins, e.g. transferrin and albumin, which may bind on the SAB surface non-specifically. These types of non-specific bindings are not usually problematic with conventional PAN-IgG detection, but interfere with lectin detection. Further cleanup of the serum is required to remove these non-DSA glycoproteins, which can be achieved with protein G column or IgG clean up column. For now, it is not clear the identity of these proteins or the influence to the conventional single antigen bead assay.

Using the lectin probe and deglycosylated SAB system, preliminary detection of glycosylations of HLA antibodies were made with positive control sera which are collections of high PRA patient's serum used as control sera for conventional DSA detection in our laboratory. Since it is a heterogeneous collection of sera from various patients, it is possible to see differential glycosylation levels in relation to the titer of HLA antibodies. Figure 2A shows the comparison of PAN-IgG detection of the positive sera and RCA lectin probe detection of the same sample. It is obvious that the differential levels of glycosylations are present in this heterogeneous sample of HLA antibodies. Figure 2B demonstrates detection of glycans on DSA or HLA antibodies from a post-transplant patient's serum. Investigation of the glycosylation profiles of the post-transplant patients are under way currently, which will include the analysis of association with DSA level, IgG subclasses, C4d biopsy, C1q and AMR [11].

With the lectin probe and simple modification of conventional single antigen bead assay, it is demonstrated that the differential glycosylations on the HLA antibodies can be monitored for clinical application. To our knowledge, no attempt has been made to correlate the glycosylation profile of DSA and the incidence of AMR or other rejection markers for allograft recipients yet, and this approach will provide valuable information about the possible effector functions of the circulating DSA. Combining with other information, such as IgG subclass analysis, C1q, C4d [11], this approach will give more comprehensive understanding of the detected DSA and its consequence in AMR which we have not completely understand yet. We postulate the methodology presented will give a more comprehensive and complete understanding of the detected AMR and the donor specific antibody *in vitro* correlate.

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