Effects of Eliciting Immune Response in Rabbits on IgG N-glycans

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Introduction

Several studies have shown that changes in serum IgG glycosylation may correlate with vaccination efficacy. In these clinical studies, it is hard to connect the altered glycosylation to the treatment and not some other factor(s). Also, the lack of experimental controls makes it difficult to assess the correlation between glycosylation profile and vaccine efficacy. Here, we examine the serum IgG glycosylation patterns from 8 rabbits, in half of which vaccination was effective, whose serum was collected before and several times after vaccination. The glycosylation pattern of the Fc region was determined by a new HILIC-SRM analysis of trypsin-digested serum, while the vaccine efficacy was monitored by ELISA.

Methods

Rabbit Vaccination
Serum samples were obtained from rabbits before vaccination (Pre-Vac) and were vaccinated immediately after (week 0). Rabbits were then boosted two, three, and seven weeks after pre-vac samples were drawn. The first test-bleed (TB1) was drawn on week five and the second test-bleed was drawn on week 8.

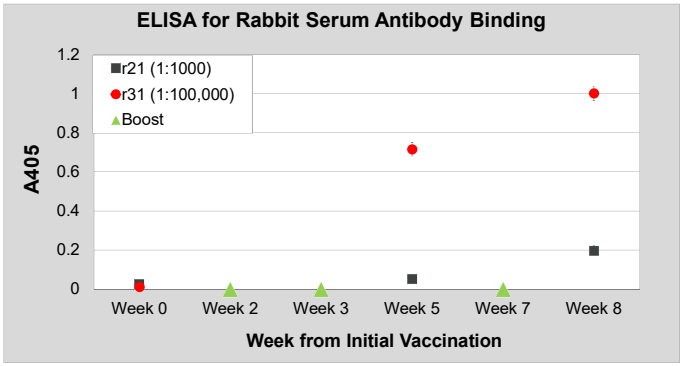
ELISA Assay
Wells of Immulon 2HB flat bottom plates were each coated with 250µg of antigen, blocked with 2µg BSA, and incubated with serum diluted with 1% BSA, 0.5% Tween-20 in PBS for 1 hr. After washing wells of the serum dilutions, 12µg of secondary antibody (Jackson !mmunoResearch Labs, 111-055-003) were aliquoted to each well and incubated for 1 hr. Secondary antibody was washed out of wells and 50µg secondary antibody substrate was added to each well. Absorbance at 405nm was measured using a SpectraMax Plus 384 MicroPlate Reader 30min after addition of substrate.

Serum Digestion
Each 25µL aliquot of rabbit serum was mixed with 50µg of iGlycoMAb (GlycoScientific), 25µL 100mM ammonium bicarbonate, and 5µL of 200mM dithiothreitol (DTT) then incubated for 1hr at 37C. A 4µL aliquot of 1M Iodoacetamide was added to each sample, which were then incubated at 1hr at room temperature. A 20µL aliquot of 200mM DTT was added to each sample, which were incubated for 1hr at room temperature. A 20µL aliquot of trypsin (2µg/µL in 50mM ammonium bicarbonate) was added to each sample, which were incubated at 37C for 18hrs. Glycopeptides were then purified from the digest using J.T. Baker Octadecyl disposable extraction columns.

LC-SRM Analysis
For the LC-SRM analysis, samples were suspended in 30µL 70% ACN in 0.1% formic acid/50mM ammonium formate/water and 5µL was injected into a Shimadzu Nexera LC using a HALO Penta-HILIC column (2.1 x 150mm, 2.7µm particle size). The gradient used was 70-60% ACN in 0.1% formic acid/50mM ammonium formate/water at a 0.6mL/min flow rate where the column was heated to 70C. SRM analysis was performed using a AB 4000 Q trap utilizing a turbo spray ion source. Three transitions were scheduled for each $[M+2H]^{2+}$ glycopeptide (sequence EQQFNSTIR) precursor ion and were scheduled with a 150s detection window.

ELISA Data

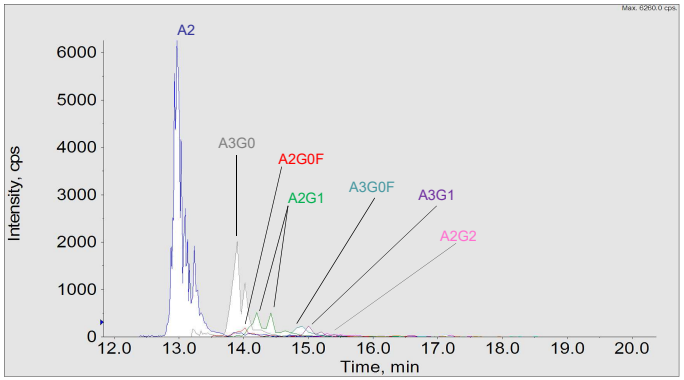
Below is a comparison between two rabbits, one which demonstrated a strong immune response to vaccination (r31) and one which did not (r21).



Note that the serum samples were diluted to different concentrations for this assay, with the non-responding serum sample being 100x more concentrated than that of the responding serum sample.

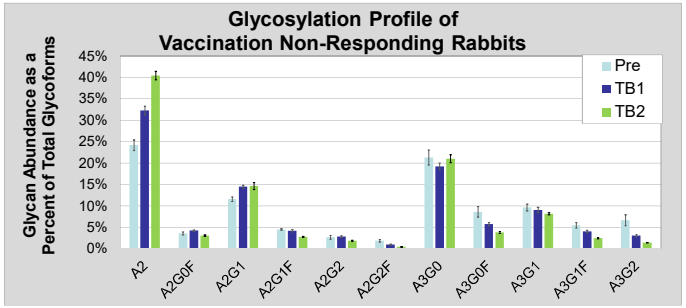
LC-SRM Analysis

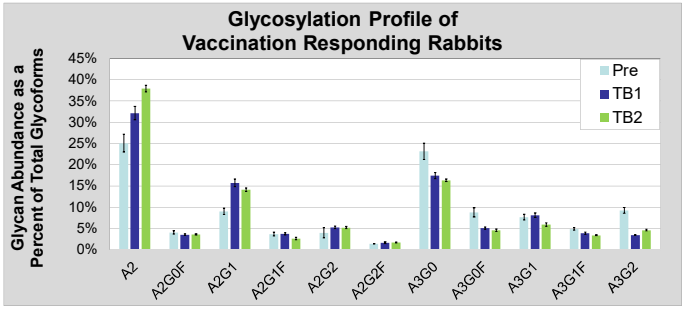
Shown below is example chromatograms of a serum digest for a rabbit which responded to vaccination (r31). Displayed are the extracted ion chromatograms for transitions corresponding to the $[hexnac+H]^+$ (204.1 m/z) fragment ion for the 11 most abundant glycoforms from rabbit serum IgG Fc glycopeptides.



Glycosylation Profiles

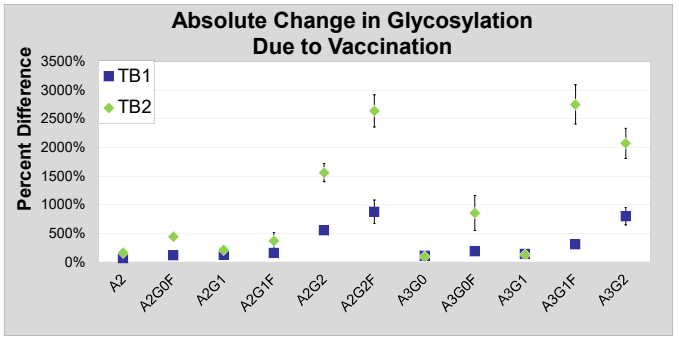
Glycosylation profiles for each serum sample were calculated by the sum of the average peak area ratios of the corresponding rabbit IgG Fc glycopeptides to the iGlycoMAb A2G0F peak area. This sum was normalized to the sum of all rabbit IgG Fc glycopeptide peak areas.

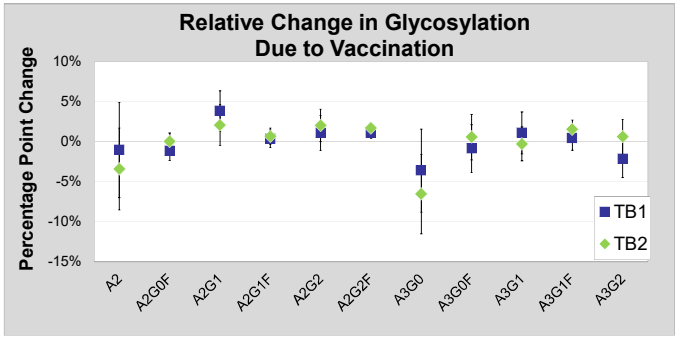




Changes in Glycosylation

The charts show the overall difference in glycosylation between the IgG Fc glycopeptides from rabbits that responded to vaccination and those that did not. The differences in pre-vaccination glycosylation were accounted for in this analysis. Absolute changes were calculated by not normalizing the data to the sum of all rabbit IgG Fc glycopeptide peak areas (as was performed in the analysis for relative changes).





Conclusions

- Relative changes in glycosylation due to vaccination were measured to significant for only one of the eleven glycoforms: an increase in the prevalence of A2G2F.
- An increase in the absolute abundance of all glycoforms was observed, with A2, A2G1, and A3G0 seeing the largest absolute gains, and A2G2F, A2G2, and A3G2 seeing the largest changes in absolute abundance.
- Absolute changes in glycopeptide abundance are more indicative of successful vaccination than relative changes.
- Future work will move towards analyzing low abundance glycopeptides, more rabbits, and the effects of sequential booster shots.

References

1. Jing-Rong Wang et. al. 2015. Scientific Reports 5:7648.
2. Anne Cathrine Vestrheim et. al. 2014. Immunity, Inflammation and Disease 2(2):76-91.
3. Rosina Plomp et. Al. 2016. Molecular & Cellular Proteomics 15:2217-2228.

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