Antibody glycomics signatures of SARS-CoV-2 infection and vaccination

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APPENDIX

ENGLISH SUMMARY

Immunoglobulin G (IgG) antibodies can exert their functions via both Fab-mediated neutralization and Fc-mediated effector functions, both of which are crucial for protective immunity in COVID-19. Importantly, effector functions and resulting inflammatory responses are impacted by the structure of *N*-glycans linked to the Fc-tail of IgG. Studying antibody glycosylation in emerging infectious diseases such as SARS-CoV-2 allows to gain insight into specific glycan signatures at the early stages of infection, and to investigate whether these reflect how the disease would progress. For example, low fucosylation is a common glyco-phenotypic signature of IgG1 produced against the spike (S) protein of severely ill SARS-CoV-2 infected patients early on in their disease course, but has likewise been described in other disease settings, where the antigen is presented in the context of host-cell membranes **(Chapter 2)**. In this thesis, antibody glycomics signatures of SARS-CoV-2 infection and vaccination have been explored using an established liquid chromatography – mass spectrometry-based method relying on affinity-isolation and proteolytic digestion of both total and anti-S IgG. In **Chapter 3**, the glycosylation of SARS-CoV-2 anti-S IgG antibodies were found to be vastly skewed relative to total IgG and to change in a highly dynamic fashion. Moreover, IgG glycosylation was shown to be an early severity marker and showed patient stratification potential, with predicting power for intensive care admission within a hospitalized patient population. Early detection of a pro-inflammatory glycosylation pattern may provide a broader intervention window and decrease the number of ICU-admissions. Furthermore, anti-S IgG1 glycosylation levels obtained with LC-MS show promise to supplement clinical parameters and biomarkers of inflammation, that have together been used for the severity score calculation of hospitalized COVID-19 patients. Similarly to SARS-CoV-2 infection, antibodies generated against the spike protein upon BNT162b2 mRNA vaccination also induced a transient afucosylated anti-S IgG1 response in antigen naïve individuals, albeit to a lower extent than in severely ill patients, exemplifying the influence of the type of immunization on antibody glycosylation **(Chapter 4)**. Upon vaccination, the observed initial, mild afucosylated response was additionally accompanied by low fucosyltransferase (FUT8) expression in antigen-specific plasma cells. Furthermore, the observed initial anti-S IgG afucosylation signature may aided mounting a stronger immune response, as indicated by its correlation with antibody amounts following the second vaccination dose. Given the impact of glycosylation on antibody function, deciphering the

underlying regulatory mechanisms influencing IgG glycosylation will be of great importance to better understand the inflammatory potential, vaccine efficacy and protective capacity of vaccine- or pathogen-induced IgG in both body fluids and tissues in the future.

In **Chapter 5** and **6**, the reaction steps of a previously developed linkage-specific sialic acid derivatization workflow were studied in more detail. Key players in such reactions are catalyst, of which novel types with different physico-chemical properties were introduced in **Chapter 5**. In **Chapter 6**, prior lactone formation was found to be a prerequisite for subsequent amidation of α2,3-linked sialic acids, which proceeds via direct aminolysis of the C2 lactone. Together, these new insights will be beneficial for the rational optimization of high-throughput (MALDI-)MS-based glycomics and glycoproteomics workflows relying on linkage-specific sialic acid derivatization.