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Digging into the Multifaceted Variability of Antibody Molecules: Fc-Proteoform Profiling Illuminates Autoimmune Responses in Rheumatoid Arthritis

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Summary

Autoantibodies and their post-translational modifications (PTMs) are insightful biomarkers of autoimmune diseases informing clinical decisions. However, current methodologies are mainly focused on IgG1 glycosylation, which represents only a subset of the IgG proteome. Quite the contrary, sequence variations, multiple glycosylation sites, and additional PTMs cumulate in structural and functional complexity of the immunologically decisive Fc-domain. To grasp this complexity, including low abundant autoantibody subpopulations, comprehensive yet sensitive methodologies are urgently needed and will allow to understand the role of (auto)antibody-proteoforms and fully unravel their biomarker potential.

Here, we present a holistic analytical approach based on isolation and analysis of the intact Fc-subunit to decipher (auto)antibody Fc-proteoforms and demonstrate its capabilities for the prototypic autoimmune disease rheumatoid arthritis (RA). To this end, Fc-subunits were obtained from anti-citrullinated protein antibodies (ACPA) via antigen-specific immunocapturing and total IgG via Fc-specific capturing. Analytical characterization of Fc-subunits required the development of a nanoscale reversed-phase HPLC approach coupled to mass spectrometry via dopant-enriched nanoESI that allowed separation of IgG allotypes and subclasses, while providing the necessary sensitivity to assess antigen-specific antibodies. Characterization of paired plasma and synovial fluid samples of RA patients revealed a clear molecular distinction of ACPA compared to total IgG besides plasma- and synovial fluid-dependent differences. Prominent changes in glycosylation included high fucosylation in ACPA and low galactosylation in synovial fluid-derived ACPA. Monitoring of hitherto neglected IgG features such as allotype ratios, C-terminal truncations, and doubly-glycosylated Fc-subunits, extended the current view of (auto)antibody complexity. Integration of this wealth of Fc-proteoforms showed a separation of patients that differed in disease activity and led to the identification of disease-associated proteoforms. Taken together, the developed methodology provided comprehensive allotype- and subclass-specific Fc-proteoform profiles surpassing state-of-the-art peptide approaches and uncovered disease-associated Fc-proteoforms of biomarker potential, thus calling for implementation of such methodologies in autoimmunity and beyond.