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Commentary

New technologies laying a foundation for next generation clinical serology

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Combinatorial protein engineering methodologies such as phage display are powerful tools for high-throughput investigations of protein-protein interactions. Since originally developed [1], phage display has become a work horse around the world both for basic protein chemistry work as well as for academic and pharmaceutical development of biotherapeutic drugs, e.g. the therapeutic antibodies used in autoimmune disease and cancer.

In the current study by Román-Meléndez et al [2], the scope has been to use phage display technology to help define the breadth of anti-citrullinated peptide autoantibodies (ACPA) in rheumatoid arthritis (RA). Within the field of clinical immunology, a positive serological antibody test is often part of the clinical diagnostic criteria, and in many disease settings the precise target(s) of autoantibodies are well understood. However, in RA synthetic peptide(s) (CCP2/3) are used in the clinical assays serving as a proxy for ACPA reactivity. The autoantigens contain the modified amino acid citrulline, which is naturally generated by enzymatic modification by PADs, but the precise autoantibody targets are still debated in RA.

Phage display has proven an effective method for epitope mapping of monoclonal antibodies as well as for understanding natural immune responses. The phage systems enable screening of a large number of candidate epitopes with more affinity discrimination than peptide arrays, but comes with the caveats of primarily binding to linear epitopes and not including mammalian specific post-translational modifications (PTMs). While randomized peptide libraries have been successfully used in many studies including recently for mapping SARS-CoV2 antibodies [3], cDNA or synthetic peptidome libraries have the advantage of only containing naturally occurring epitopes making them more applicable for directed human serological studies. Many original phage display platforms have used the M13 bacteriophage, yet the T7 phage-based systems have recently shown to be highly competitive, in particular for fast high-throughput proteomic screenings. This has previously also been demonstrated using the VirScan platform for screening of antibody responses to common viruses [4]. In Román-Meléndez et al [2], this concept is further advanced by developing a T7 proteomic platform for mapping autoantibody responses to PTM antigens targeted by ACPA in RA.

The authors have used a human peptidome phage library which could be thought of as a synthetic cDNA library covering the entire human proteome with peptides and have added PAD enzymatic modification to create citrulline epitopes that could be targeted by RA autoantibodies. Indeed, the T7 phage display set-up has been successfully applied to identify (non-PTM) antigens in autoimmune diseases such as myositis, multiple sclerosis, rheumatoid arthritis, sarcoidosis and diabetes [5-7].

Importantly, mapping human autoantibody responses provides several challenges, especially in systemic and rheumatic diseases where immune complexes are regarded as important contributors. In these diseases, autoantibodies could bind to non-protein, cryptic or PTM epitopes. One would also expect rather low affinity binding and a certain level of multi-reactivity and possibly also unspecific stickiness compared to other antibody responses. These aspects need to be taken into consideration as the field advances.

In RA, ACPA have been found to have several unusual features, including extensive multi-reactivity to citrullinated peptides from a range of proteins. Studies of monoclonal ACPA have demonstrated that the autoantibodies are highly selective but still capable of binding up to thousands of different autoantigens by recognition of short citrulline consensus epitopes consisting of only a few critical amino acids shared between the proteins [8]. Notably, each clone has a distinct recognition motif, however glycine adjacent to the citrulline seem particularly common as well as threonine and serine [8,9]. Interestingly, glycine is both common in un-structural parts of proteins that may be accessible for enzymatic modification and in proposed PAD-enzyme substrate motifs implicating how PAD-citrullination may drive the ACPA response.

Importantly, when analyzing polyclonal serum responses, you have to consider both the heterogeneity of different antibody clones and the diversity of multireactivity profiles in individual clones. Similarly, in analysis of PAD-modifications, if human derived antibodies are used in the detection, you have to carefully consider the antibody recognition profiles. The complexity is further increased by the fact that autoreactivities to additional PTMs can be detected in RA serum [10] and certain ACPA clones besides binding citrullinated peptides

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also targets acetylated and homocitrullinated epitopes with similar or higher affinity [8]. Altogether, this considerably magnify the challenges in identifying critical autoantigens driving RA pathogenesis, if such an individual autoantigen even exists.

The Román-Meléndez study provides a proof-of-concept for future studies of antibody responses to modified proteins, as such immune reactions may be more prominent then so far appreciated, simply because no one has looked close enough. While many challenges withstand in studying PAD enzyme modifications and autoreactivity to PTMs in RA, we foresee that the current report will inspire studies also of other protein modifications in RA as well as in many other disease settings.

Contributors

CG conceptualization, writing original draft, reeview & editing. VM writing original draft, review & editing.

Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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