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Review

Secreted autoantibody repertoires in Sjögren's syndrome and systemic lupus erythematosus: A proteomic approach

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ABSTRACT

The structures of epitopes bound by autoantibodies against RNA–protein complexes have been well-defined over several decades, but little is known of the clonality, immunoglobulin (Ig) variable (V) gene usage and mutational status of the autoantibodies themselves at the level of the secreted (serum) proteome. A novel proteomic workflow is presented based on affinity purification of specific Igs from serum, high-resolution two-dimensional gel electrophoresis, and de novo and database-driven sequencing of V-region proteins by mass spectrometry. Analysis of anti-Ro52/Ro60/La proteomes in primary Sjögren's syndrome (SS) and anti-Sm and anti-ribosomal P proteomes in systemic lupus erythematosus (SLE) has revealed that these antibody responses are dominated by restricted sets of public (shared) clonotypes, consistent with common pathways of production across unrelated individuals. The discovery of shared sets of specific V-region peptides can be exploited for diagnostic biomarkers in targeted mass spectrometry platforms and for tracking and removal of pathogenic clones.

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Abbreviations: aa, amino acid; D, diversity; 2-DE, two-dimensional gel electrophoresis; H-chain, heavy chain; HCDR3, heavy chain complementarity determining region 3; HPLC, high-performance liquid chromatography; Ig, immunoglobulin; J, joining; L-chain, light chain; MRM, multiple reactions monitoring; MS, mass spectrometer; NGS, next generation sequencing; Q-TOF, quadrupole-time of flight; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; V, variable.

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1. Introduction

Autoantibodies play a key role as serum biomarkers in systemic autoimmune diseases and remain an important entry point for basic and clinical research into human autoimmunity [1]. These can be divided into two broad groups: "classical" autoantibodies directed against RNA–protein complexes such as Ro/La, Sm/RNP and ribosomal P that are present in high serum concentrations, have good disease specificity and are markers of future disease and are generally detectable by standard solid-phase immunoassays [2,3]; and "functional" autoantibodies against cell surface receptors and ion channels such as M3-muscarinic receptors in primary Sjögren's syndrome (SS) that are of low concentration, directly pathogenic following passive transfer, difficult to detect by standard immunochemical methods and often require sensitive physiological assays for their detection [4]. Irrespective of their targets and biological effects, new approaches are required for the detection and molecular characterisation of human autoantibodies in systemic autoimmunity.

Most studies of humoral autoimmunity to date have focussed on the structural and biochemical characteristics of the target autoantigens and identification of their autoepitopes, with the hope of finding more clinically relevant biomarkers [5–7]. Related areas of research include disease specificity of apotopes (epitopes expressed on the surface of apoptotic cells) and human and murine studies on HLA class II phenotype and determinant spreading [8,9]. Despite considerable progress in identifying the amino acid (aa) sequences of linear and conformational epitopes in human systemic autoimmune diseases, such studies tell us nothing about the molecular characteristics of the autoantibodies themselves, in particular their clonality, immunoglobulin (Ig) variable (V) gene usage and mutational status. Analysis of serum autoantibody repertoires, if technically feasible, is likely to provide key insights into the emergence of autoreactive B cells in systemic autoimmunity, as well as leading to strategies aimed at removing these B-cell clones.

Although antibodies were discovered by Behring and Ehrlich at the end of the nineteenth century, little is known about the size and molecular composition of the actual secreted (serum) antibody repertoire in humans. These questions are beginning to be addressed by a combination of next generation sequencing (NGS) of B-cell repertoires and antibody proteomics [10–17], although progress remains slow. Here, we review recent work on the proteomic analysis of serum autoantibodies in systemic lupus erythematosus (SLE) and primary SS based on mass spectrometric sequencing of serum Igs, and discuss how this new field of autoantibody research may lead to new diagnostic and therapeutic approaches in systemic autoimmune diseases.

2. A proteomic workflow for molecular characterisation of secreted (serum) autoantibodies

As discussed above, little is known about the clonality and V-region structures of systemic human autoantibodies. Early studies using two-dimensional gel electrophoresis (2-DE) suggested that anti-Ro60 and anti-La humoral responses were oligoclonal [18,19], but amino acid (aa) sequencing of heavy (H) and light (L) V-regions of these putative clonotypic populations was not thought feasible because of the diversity of humoral responses and interference from normal serum immunoglobulins. We have overcome these limitations by developing a proteomic approach for determining molecular signatures of serum autoantibodies, based on positive selection of polyclonal sera on defined antigenic determinants followed by high-resolution 2-DE and de novo and database-driven mass spectrometric sequencing.

The workflow begins with an autoantibody purification step from a human autoimmune serum followed by 2-DE and mass spectrometric sequencing and bioinformatics (Fig. 1). We initially obtained purified autoantibody for sequencing by passing immune sera over affinity columns expressing recombinant antigen fragments or the complete autoantigen [20–22]. This labour-intensive step has now been superseded by a simple ELISA plate-based elution method that dramatically reduces serum and autoantigen requirements and workflow times [23,24]. Furthermore, the Orbitrap mass spectrometer used in earlier studies has been superseded by a high-end high-performance liquid chromatography quadrupole-time of flight mass spectrometer (HPLC Q-TOF MS) that performs higher MS/MS mass accuracy protein sequencing and improved protein coverage [23,24]. While these improvements have shortened purification and sequencing steps from weeks to days, and can be applied to any immune serum, sample size remains limited by the complexity of Ig bioinformatics analysis. In particular, the methodology rarely obtains full sequence through the diversity (D) region of the H-chain and complete H-chain complementarity determining region 3 (HCDR3) sequence is rarely achieved by de novo sequencing, compounded by the unavailability of HCDR3 sequences in databases. New developments in de novo sequencing of HCDR3 regions in conjunction with immunoproteogenomics approaches may overcome this limitation [25]. It has not yet been possible to sequence functional autoantibodies with this methodology because of their low serum concentration and lack of binding to antigen in the solid phase.

An additional benefit of direct sequencing of serum autoantibodies is that H- and L-chain constant regions can be identified with complete accuracy at the level of aa sequence, as opposed to standard immunochemical methods that depend upon on the specificity of anti-L-chain antibodies. Early data on kappa/lambda restriction of autoantibodies, based on conventional immunochemistry, may need to be revised in light of this new technology [23]. Other advantages include the ease of serum collection, stability of antibodies under storage, small volume requirements (a V-region peptide signature can be obtained on as little as 1 ml of sera) and applicability to archival sera.

The review will now describe recent advances in the molecular characterisation of classical RNA–protein autoantibodies in primary SS and SLE at the level of the serum proteome. Direct autoantibody sequencing has revealed for the first time that humoral autoimmunity in these diseases is dominated by public (shared) sets of clonotypic autoantibodies.

3. Secreted Ro/La autoantibody repertoires in primary Sjögren's syndrome

3.1. Anti-Ro60

Ro60 is considered to be a primordial autoantigen involved in the initiation of systemic autoimmunity, with anti-Ro60 occurring years before clinical autoimmunity and becoming pathogenic on binding Ro60 on the surface of apoptotic fetal cardiomyocytes in congenital heart block [2,26,27]. A subset of anti-Ro60 in patients with linked anti-Ro60/La responses react on solid phase immunoassay with an apical peg-like structure on Ro60 that is expressed on intracellular Ro/La–RNP complexes [28]. In an initial 2-DE step to assess the clonality of affinity column-purified anti-Ro60 peg autoantibodies, we were surprised to find an identical H- and L-chain monoclonal pattern in all patients. Mass spectrometric sequencing of individual gel spots revealed an IgG1 kappa-restricted monoclonal Ig specified by an IGHV3-23/IGKV3-20 chain pairing signature that is shared (public) among unrelated patients [21]. The V-regions of each chain were expressed both as germline-encoded and somatically mutated peptides, although it remains unclear as to whether shared aa replacements originate from positive selection or underlying genetic variation.

The discovery of this stereotypic monoclonal autoantibody directly against a bona fide systemic autoantigen argues against a random selection mechanism of autoantibody production. As pointed out in a recent editorial [29]: "the fact that there is such an astonishing similarity between patients powerfully suggests that the entire pathway from original stimulus through the mechanism that generates autoimmunity must be virtually identical in its molecular details from patient to