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Human peptidome display

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Abstract

A peptide library representing the entire human proteome is applied to the discovery of autoantigens.

Autoimmune diseases arise when the immune system misrecognizes the ‘self’ as foreign and attacks the body’s own tissues. In most cases, the target molecules that trigger these self-destructive immune reactions are unknown, impeding the development of therapies and diagnostic tests. In this issue, Larman *et al.*¹ describe a powerful new approach to autoantigen discovery that is based on the first synthetic representation of a complete human proteome. The authors identify novel autoantigens in individuals with a neurological autoimmune disease and also show how their method can identify protein-protein interactions unrelated to antibodies. Knowledge of the specificity of autoantibodies is important as it may allow the development of antigen-specific treatments or provide mechanistic biomarkers to streamline clinical trial design.

A major obstacle to antigen discovery is the paucity of technologies that enable large-scale, unbiased surveys of the vast universe of targets that antibodies can recognize. These targets include small domains on proteins (including post-translational modifications with carbohydrates and lipids) as well as carbohydrates and lipids that are not associated with proteins. One of the least biased technologies for autoantigen discovery is mass spectrometry. In this approach, lysates are generated from the diseased tissue, immunoblotting or immunoprecipitation is carried out with the patient’s serum as the source of antibodies, and mass spectrometry identifies the bound autoantigens. Autoantigens targeted in systemic lupus erythematosus and rheumatoid arthritis were discovered in this way². Although mass spectrometric methods allow *de novo* discovery of a wide range of autoantigens, including those that are post-translationally modified, they are somewhat limited by biases in selection and preparation of tissue samples.

In array-based approaches, the reactivity of antibodies in patient samples is tested against panels of previously identified autoantigens arrayed on surfaces³. Antigen arrays also allow for screening of autoantibodies and other proteins for reactivity against polypeptides expressed using phage, bacterial or mammalian cell-based cDNA and peptide expression libraries^{4,5}. Recently, this approach was extended to characterize reactivity against libraries of random synthetic peptoids, *N*-substituted glycines whose side chains are appended to the

nitrogen atom of the peptide backbone, enabling identification of antigenic shapes⁶. Although powerful and less biased than antigen arrays, peptoid arrays require substantial follow-on experimentation to identify the actual autoantigens.

Another traditional approach to autoantigen identification relies on expression cloning with cDNA libraries. Polypeptides encoded by the cDNAs are screened for reactivity with patient-derived autoantibodies. Although several autoantigens have been identified using this technique^{7,8}, current methods are limited by low rates of expression of in-frame coding sequences and inefficiencies in analyzing binding interactions.

The new technology developed by Larman *et al.*¹, called phage immunoprecipitation sequencing (PhIP-Seq), combines phage display of a synthetic human peptidome with immunoprecipitation and high-throughput sequencing (Fig. 1). The authors begin by synthesizing >400,000 oligonucleotides encoding 36-amino-acid peptides that cover all open reading frames in the human genome. The oligos are then packaged in phage for display on the phage surface. Phage expressing candidate autoantigens are immunoprecipitated using antibodies present in patient samples and identified by high-throughput DNA sequencing.

Using this system, the authors analyze autoantibodies in the spinal fluid of three patients with paraneoplastic neurological autoimmune disease. Paraneoplastic syndromes are unique diseases at the interface of autoimmunity and tumor immunity. In these rare syndromes, the body produces autoantibodies that recognize molecules present both in a tumor and in normal brain tissue⁷, leading to an autoimmune reaction. Larman *et al.*¹ identify previously described autoantigens and new candidate neurologic autoantigens in two patients who had clinical features of paraneoplastic disease but tested negative for antibodies against a panel of established paraneoplastic disease-associated antigens. Naturally, further testing and validation of the new putative autoantigens will be necessary to assess their role in pathogenesis and clinical utility. In principle, however, validated autoantigens could be used to design therapeutic blocking antibodies against domains in the antigens that are expressed in the tumor but not in normal tissue.

Detection of autoantibodies represents a central component of the diagnostic criteria for rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis and Graves' disease, and elucidation of the autoantigens in additional autoimmune diseases is expected to enable development of tests to detect autoantibodies with diagnostic utility. Although multiple programs are attempting to develop antigen-specific therapeutics, to date no therapy using antigen-specific tolerance is approved for the treatment of any human autoimmune disease.

In addition to identifying antigens, PhIP-Seq can also be used to characterize a variety of biomolecular interactions. The authors show this by identifying proteins that interact with protein replication factor 2. In another variation of the approach, it should be possible to screen for small molecules that modulate protein-protein interactions. Finally, by designing a different peptidome based on a microbial genome, one might adapt the approach to identify immunodominant epitopes present in microbes.

Peptidome phage display has several limitations. First, it cannot include post-translational modifications. Certain post-translational modifications are known to be critical targets of autoimmune responses, including protein citrullination in rheumatoid arthritis and protein cleavage in systemic lupus erythematosus⁹. Second, 36-amino-acid peptides expressed on the surface of phage cannot display conformational structures that exist only in native, full-length proteins. Certain tertiary and quaternary protein structures have been identified as the targets of auto-antibodies that mediate autoimmune tissue injury and of neutralizing antimicrobial antibodies. For example, in the autoimmune neurologic condition known as stiff-person syndrome, the autoantibody response recognizes conformational tertiary

epitopes on glutamic acid decarboxylase¹⁰. Peptide sequences expressed on the phage surface in nonnative conformations have the potential to bind antibodies in a nonphysiological manner. Finally, alternative splicing variants could act as autoantigens, and could be incorporated in the next generation of peptidome phage-display libraries.

Despite these limitations, PhIP-Seq is a comprehensive, powerful approach for auto-antigen discovery as it combines a synthetic representation of the human proteome with high-throughput sequencing. We anticipate that it will be fruitful to apply this approach to investigate a spectrum of autoimmune diseases, including multiple sclerosis, psoriasis and Crohn's disease.

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Figure 1.

Phage immunoprecipitation sequencing for autoantigen discovery. Larman *et al.*¹ construct a T7-phage peptide library to express 413,611 DNA sequences that encode overlapping 36-amino-acid peptides spanning the 24,239 polypeptides encoded by all of the open reading frames in the human genome. The phage are then mixed with autoantibody-containing biological fluid from patients with an autoimmune disease. Phage expressing a 36-aminoacid peptide bound by a patient autoantibody are immunoprecipitated using beads coated with Protein A and Protein G. Finally, high-throughput sequencing of the human DNA carried by the immunoprecipitated phage identifies the 36-amino-acid peptide and, thus, the putative autoantigen.