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# Phage display identification of immunodominant epitopes and autoantibodies in autoimmune diseases

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Phage display represents an invaluable tool to study autoimmune diseases. The side effects of immunosuppressive drugs for the treatment of autoimmune diseases raise awareness of the need to explore alternative therapeutic approaches such as antibodies and peptides. Therefore, phage display is an important technique for generating such molecules, so the purpose of this review is to determine the potential advantages of this technique in the research of autoimmune diseases. Many studies have also demonstrated the efficacy of phage display in identifying immunodominant epitopes of autoimmune diseases such as Goodpasture disease, immunologic thrombocytopenia, and systemic lupus erythematosus. Phage display peptide libraries have been screened with immunopurified autoantibodies from patients with autoimmune diseases. This makes it possible to more precisely locate the autoantibody binding sites, reveal a possible epitope sharing between the host and microbe, and identify a motif that mimics an antigenic structure such as that of dsDNA. Several studies have been conducted that have investigated the effectiveness of phage display in isolating autoantibody repertoires of autoantibodies against human epitopes. This allows the identification and design of antibody fragments (e.g., Fab, scFv, sdAb) that could block the binding of autoantibodies such as the deposition of IgG in the kidney and reduce the clinical signs of disease. In conclusion, phage display helps identify common epitopes and hotspot residues that can be potential therapeutic targets for the treatment of autoimmune diseases. This leads to a better understanding of the immunopathogenesis of autoimmune diseases and the development of more specific therapeutic strategies.

**Keywords:** phage display, autoantibody, autoimmune disease, epitope, peptide library, antibody library.

## Introduction

Autoimmune diseases are a leading cause of morbidity and death in humans, affecting around 4.5% of the population (1). The inability of the immune system to "tolerate" self molecules can lead to systemic or organ-specific damages (2). Autoimmunity can be induced or triggered by many factors (3) (e.g. genetic, sunlight exposure, etc.) including microorganisms (4,5). Autoantibodies are frequently considered a clinical marker of autoimmune disorders (6). Conventional therapies against autoimmune diseases are

based on immunosuppressive drugs that globally attenuate the immune system. While these reduce symptoms to some degree, they may have serious side effects (7). Thus, other therapeutic agents such as antibodies and peptides are being currently investigated since they are more specific and less immunogenic. To achieve this, we need to know the autoantibodies associated with each specific autoimmune disease and their targets. There are now some systems (e.g. phage display) available that are

optimized for this purpose. Phage display is an efficient system of selection of antibodies or peptides having a specific binding capacity among a large number of variants (8). The purpose of this review is therefore to gain an understanding of the existing research of phage display and determine their potential advantages in studying autoimmune diseases.

## Sources of information

An extensive literature review was conducted in PubMed's databases on autoimmune diseases and autoantibodies in combination with the keyword phage display.

## Phage display identification of immunodominant epitopes in autoimmune diseases

A thorough understanding of epitopes would help develop therapies to selectively neutralize autoantibodies and establish the basis for research to identify the etiology of autoimmune diseases. Several typical examples of such an approach are described in the literature, however, we will focus on three fields of applications.

One field of application of phage display includes for example epitope mapping. Jones *et al.* used phage display technology to more precisely localize the circulating Goodpasture autoantibody binding sites (9). The Goodpasture epitopes have been localized in the two binding regions ( $\alpha$ 317–31 of EA and  $\alpha$ 3127–141 of EB) of  $\alpha$ 3(IV)NC1 domain (10–12) of type IV collagen. Critical amino acid residues (Ala18, Ile19, Val27, and Pro28) were identified in EA using recombinant chimeric proteins (13). Two random phage display peptide libraries from New England Biolabs (PhD-c7c PhD-12) were screened with immunopurified circulating autoantibodies from a patient with Goodpasture disease. The selected phage sequences with site-directed mutagenesis and surface plasmon resonance identified new residues (Thr26 and Tyr30 of EA; EB region (Thr127, Pro131, His134, and Lys141 of EB; and Thr99 and Ala196 outside EA/ EB) involved in autoantibody binding. From this, it can be concluded that the phage display approach can also identify new peptides to neutralize the binding of circulating Goodpasture autoantibody to the autoantigen *in vivo*.

Another field of application of phage display is to investigate molecular mimicry. Li *et al.* investigated whether sharing of epitope between host and HIV may be responsible for the immunodominant epitope in HIV-1-immunologic thrombocytopenia (14). A phage display 7-mer peptide library was screened for peptides reactive with a rabbit as well as human anti-GPIIIa49-66 antibody. The selected peptides were then analyzed for their capacities to inhibit human anti-GPIIIa49-66-induced platelet oxidation/fragmentation and compared to antibody inhibition by its immunodominant epitope (GPIIIa49-66). Affinity-purified human anti-GPIIIa49-66 antibodies isolated from HIV-1-infected thrombocytopenic patients were capable of selecting peptides with similar sequence identity to HIV-1 proteins (env, pol, nef, gag). Synthesized HIV-1 mimicry peptides inhibited antibody-induced platelet oxidation/fragmentation and anti-HIV-1 peptide antibodies caused thrombocytopenia in mice in the same way and intensity as human anti-GPIIIa49-66. This finding indicates that molecular mimicry can be a contributing factor in autoimmune disease in HIV-1 infection.

The third field of application of phage display comprises the identification of mimotopes. Sun *et al.* managed to identify a peptide pattern that could mimic the antigenic and immunogenic epitope of double-stranded DNA in systemic lupus erythematosus (SLE) (15). Anti-dsDNA and anti-ssDNA antibodies can bind glomerular antigens, forming immune deposits which are considered an important step in kidney damage of SLE patients. A 15-mer peptide library displayed on gene VIII was screened with immunopurified anti-dsDNA antibodies obtained from human sera from SLE patients. The identified peptide sequence could be recognized by 88% of anti-dsDNA antibody-positive sera from SLE patients. Also, it induced the production of rabbit antibodies which cross-reacted with the peptide, ssDNA, and dsDNA. The binding of the antibodies and the peptide could be inhibited by ssDNA and dsDNA, suggesting a motif that mimics an antigenic structure of dsDNA. This peptide had not yet been tested *in vivo* to decide whether it is capable of inhibiting the deposition of autoantibodies in the kidney. Phage display peptide libraries provide a powerful technique to select reactive antigens for specific autoantibodies. However, dsDNA mimotope identification and application is probably the right approach in the diagnosis and treatment of SLE. Besides, this provides a unique opportunity to study the antigenic structure of DNA and the interaction of DNA with anti-DNA antibodies. Blocking the

deposition of anti-DNA antibodies in the kidneys is a great alternative to the relatively nonspecific cytotoxic agents used for the treatment of SLE. However, further studies need to be done regarding the variety of the binding sites of the anti-DNA antibodies that are usually deposited in the kidneys.

## Phage display identification of autoantibodies against pathogenic epitopes

Many studies have explored the efficacy of phage display in isolating the autoantibody repertoires of autoantibodies against human epitopes.

For example, Payne and colleagues isolated the repertoires of human anti-Dsg antibodies from patients with active mucocutaneous pemphigus vulgaris using phage display (16). All isolated scFvs bind to Dsg, but only some of them were pathogenic. The pathogenicity was measured by injecting the mAbs into the culture of normal human skin or by passive transfer to neonatal mice. A consensus amino acid sequence (D/E-x-x-x-W) was found in the heavy chain complementarity-determining region 3 (H-CDR3) in six of nine pathogenic antibodies. Also, site-directed mutagenesis revealed that the replacement of amino acid residues in this sequence, especially the tryptophan, could block pathogenicity but not necessarily the binding (17). The pathogenicity is due to direct antibody interference with Dsg adhesion, probably by binding to the W2 acceptor pocket of Dsg, important for its adhesion to cadherin (18,19). In summary, phage display was useful to isolate the repertoires of antibodies against Dsg to find sequence homology between them. Common epitopes in Dsg and hotspot residues can be identified which could be potential therapeutic targets to treat autoimmune diseases.

Another example is the study carried out by Wang *et al.* where they successfully generated antibodies against the main pathogenic epitope (COL17 NC16A) for autoantibodies in bullous pemphigoid (BP) (20). They combined the heavy- and light-chain genes amplified from antibody repertoires of two BP patients to construct phage display libraries which were subsequently screened against NC16A. Epitope mapping studies indicated that the isolated recombinant Fabs bind different but close or overlapping epitopes on the NC16A

domain. Only Fab-B4 and Fab-19 could inhibit the binding of autoantibodies from patients with BP disease to COL17 *in vitro*, both in an inhibitory ELISA and on skin sections. Also, they inhibited the deposition of BP antibody-activated C1q and C3 at the DEJ in human skin. None of the three Fabs were able to induce clinical signs of BP or histopathological manifestations of BP in a mouse model, however, BP autoantibodies prepared from BP patients induced the disease. Also, these results showed that the selected Fabs were not pathogenic to the mice. Only Fab-B4 and Fab-19 seem to completely block the clinical signs and stimulation of the complement C1q and C3 mediated by BP autoantibodies (20). In conclusion, it was possible with phage display to amplify the repertoire of antibodies against the COL17 NC16A domain from patients with active BP. It was identified Fabs that block the binding of autoantibodies and reduce the clinical signs of BP. This has a therapeutic potential that makes it likely to apply a more specialized treatment for BP or similar diseases.

The effectiveness of the phage display technique has been exemplified in a report by Vugmeyster *et al.* They selected a group of blocking antibodies against the human and mouse receptors for interleukin 21 (IL-21), a cytokine associated with the pathogenesis of many kinds of autoimmune diseases (21). They constructed a scFv library based on the variable (V) genes isolated from human B-cells from 160 donors (22) and screened it against purified recombinant IL-21 receptor (IL-21R) or expressed by BaF3 cells. A batch of scFvs that recognized human IL-21R and inhibited its binding to IL-21 was selected from this library. From this, 18A5 was the scFv with the strongest blocking activity of IL-21-dependent proliferation of human IL-21R transfected BaF3-, TF1-, B- and T-cells. Further, phage display libraries of variants of 18A5 were constructed by blocking six adjacent codons in the CDR3s in both VH and VL, substituted with NNS randomized codons. Of these libraries, two scFvs fragments (Ab-01 and Ab-02) with only four amino acids differences in VL CDR3 (MSRSIWGNPHVL vs. VARSNKGPHVL) had the strongest potency of neutralization in cell-based assays. However, Ab-01 and Ab-02, regardless of their similarities, showed distinct pharmacokinetics and pharmacological properties. Also, Ab-01, but not Ab-02, decreased anti-dsDNA antibody titers and the amount of anti-IL-21R antibodies deposited in the kidneys of MRL-Fas<sup>lpr</sup> mice. Untreated mouse strain developed an autoimmune disease that resembled the systemic lupus erythematosus. The effect of Ab-01 was even more remarkable in rodents and cynomolgus

monkeys. The reduced blocking effect of Ab-02 was probably due to the consequence of its fast elimination in rodents and monkeys (21). In summary, neutralizing scFvs of the IL-21 and IL-21R interaction were selected by phage display. These scFvs can prevent IgG deposition in the kidney in animal models; therefore, they can be considered potential therapeutic molecules to treat systemic lupus erythematosus.

## Discussion

The adverse effects of immunosuppressive treatment in patients with autoimmune diseases make us aware of the necessity of techniques to help us to generate alternative therapeutic molecules. This review highlights the potential applications of phage display in autoimmune diseases. The phage display is a useful technology to identify pathogenic binding sites implicated in autoimmune diseases. Peptides that resemble pathogenic epitopes and autoantibodies against such epitopes can be isolated using this technique. Chimeras from selected binders can be generated and displayed on phages to find amino acid residues involved in autoantibody binding and more precisely define the epitope. Phage peptide libraries can be screened to examine whether the epitope sharing between the host and the microbe may be involved in this immunodominant epitope. Peptide libraries are useful to find mimotopes also sequences that resemble epitopes on human or microbes proteins. Also, mimotopes can even be found when the etiological agent of the disease is unknown. Such mimotopes could inhibit the binding of the main fraction of autoantibodies in the serum of autoimmune patients. Phage displayed peptide libraries are useful to select peptide-mimotopes able to hinder the pathogenicity of the autoantibodies; therefore, they have a useful therapeutic application for autoimmune diseases. Peptide libraries could be used to identify peptides that react with the anti-DNA antibodies that are deposited in glomeruli such as in systemic lupus erythematosus patients.

Phage display was used to characterize autoantibodies (e.g. anti Dsg) isolated from patients with autoimmune diseases. This leads to an increase in our knowledge of the immunopathogenesis of these diseases and how to apply more specific and different therapeutic approaches. Phage display applies to isolate the repertoires of antibodies against Dsg1 and find similarities among pathogenic autoantibodies from pemphigus patients. These

autoantibodies seem to share sequence homology in the H-CDR3 region and recognize similar epitopes in Dsg. However, hotspot residues in the Dsg-antibody interaction could be blocked with potential therapeutic targets such as small molecules or peptides. A phage display approach was used to produce Fabs with therapeutic applications that made it possible to apply a more specific treatment for BP or similar diseases. Also, subdomain 2 is the main epitope identified by both IgG and IgE autoantibodies from patients with BP disease, making it an excellent therapeutic target. Furthermore, with phage display, a panel of neutralizing antibodies against IL-21R was generated from which one that significantly decreased IgG deposition in the kidney in animal models was selected. This may contribute to an effective therapy for lupus and in diseases related to IL-21.

A useful example of a therapeutic recombinant antibody fragment approved by the FDA indicating that these types of molecules work in humans Certolizumab pegol (23). This is a Fab fragment against tumor necrosis factor alpha (TNF $\alpha$ ) which is used for treatments of Crohn's disease, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis.

In conclusion, we can disturb the interaction between autoantibodies and epitopes with molecules that mimic the autoantibody antigen-binding sites or the epitopes on the autoantigens. There is no doubt about the phage display potential to generate such molecules. Also, phage display is a cutting-edge technology that we can use to discover antibodies and peptides that need to treat autoimmune diseases.

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