Review

Using Phage Display in Autoimmunity Research

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Abstract

Autoimmune diseases affect approximately 3% of the population and are a significant cause of morbidity and mortality. Therefore, they are the focus of vivid research aimed at the delineation of pathology, more efficient diagnosis and therapy. Phage display is a simple methodology markedly useful in studying protein-protein interactions which are the driving forces of (patho)physiological processes, including autoimmunity. The commercially available phage display peptide libraries allow the characterization and identification of antibody-antigen and receptor-ligand binding sites, thus providing basic insight into the pathological network. Through the phage display of antibody's, receptor's or ligand's protein domains it is possible to produce various self-specificities that have already been proven useful in the therapy of autoimmune diseases. Phage antibody display libraries are able to reprint the *in vivo* autoimmune response which significantly facilitates structural and functional analyses of auto-reactive antibodies and assessing their role in disease pathology.
The aim of this review was an overview of the versatile applications of phage display technology to determine their potential advantages in studying autoimmune diseases and to critically categorize and analyze the usefulness of phage displays towards understanding pathology and towards development of improved diagnostic tools. Above all its focus was on targeted therapy.

Keywords: Phage display, protein-protein interactions, autoimmune disease, antibodies, auto-antigens, structural and functional analyses

1. Introduction

Autoimmune disease is a debilitating pathology in which the body starts to attack its own organs. This is caused by an aberrant immune response that lacks self-tolerance or self-control. The immune response is mediated by regular effectors mechanisms which include antibodies, $CD4^+$ T cells, $CD8^+$ T cells, macrophages and other phagocytic and mast cells. In some autoimmune diseases (multiple sclerosis (MS) or type 1 diabetes (T1D)) the damage is caused by auto-reactive T cells, while in others (systemic lupus erythematosus (SLE)) auto-antibodies are the ones inducing the injury by either forming damaging immune complexes or binding to receptors and modulating their response.[1,2] Irrespective of the effectors involved, the immune responses are mediated by an array of protein-protein interactions between auto-antibodies, self-antigens, cell receptors and ligands. Biochemical knowledge of interactions is of utmost importance for understanding the background of autoimmune processes.

Phage display is a very applicable molecular technology for studying protein-ligand interactions and the identification of ligands for various targets. The technology is based on the ability to express a foreign (poly)peptide on the surface of bacterio(phage), a virus that infects prokaryotic cells. The most commonly used bacteriophage for polypeptide display is filamentous phage composed of ss-DNA genome packed into capside consisting of several copies of five different proteins (pIII, pVI, pVII, pVIII and pIX).[3,4] The display of foreign peptide on filamentous phage was first presented in 1985 by Smith, who fused a cDNA fragment encoding the foreign polypeptide with the gene encoding the pIII protein.[5] Since then many pha-

ge vectors, phagemid vectors and alternative display formats have been developed.[6,7] By these cloning vehicles collections of phages (libraries) displaying variants of random peptides, polypeptides of arbitrary lengths, foreign proteins including antibody fragments, and even synthetic compounds can be constructed.[6,8] Clones displaying polypeptides with affinity for a specific target can be isolated by sequential affinity selections (biopanning) of the phage library on that specific target. Biopanning involves: i) incubation of phage library with the immobilized target, ii) removal of nonbinding clones by multiple washing steps and iii) recovery of bound phages by elution. Recovered clones are then reinfected into bacteria and amplified for further 3–4 rounds of biopanning. After the affinity ranking of selected (target binding) clones, the high affinity interacting clones are identified by sequencing the inserted DNA fragments.[8,9]

The aim of this review was an overview of the versatile applications of phage display technology to determine their potential advantages in studying autoimmune diseases and to critically categorize and analyze the usefulness of phage displays towards understanding pathology and towards development of improved diagnostic tools and targeted therapy. In the reviewed literature different approaches, types of libraries (peptide, protein and antibody) and sources of studied materials (human, animal and synthetic) were used for various study purposes, such as structural analyses of disease implicated molecules, discovery of diagnostic reagents and therapeutics.

We are presenting data organized from the point of view of the library type used for studies and the scopes of the studies: structural characterization of molecules implicated in diseases, identification of auto-antigens and the search for novel therapeutic approaches.

2. Peptide and Protein Phage Display Libraries

Individual autoimmune disorders are usually accompanied by a heterogeneous group of auto-antibodies, some pathogenetically relevant and others merely bystanders of the ongoing disease. By identifying their targets (auto-antigens) one can acquire better understanding of disease pathogenesis (elucidate triggers for auto-antibody outbursts) or new candidates for disease diagnosis and therapy. Similar acquisitions can be obtained by characterizing and identifying specific peptide/protein binders of mediators and cell surface receptors involved in initiation and/or progression of autoimmune diseases. Phage display technology greatly contributed to the identification and/or characterization of self-antigens targeted by autoimmune antibodies and peptides/proteins modulating the disease mediating pathways.

Roughly, there are three different formats of (poly)-peptides displaying libraries (in reference to the length of the displayed polypeptide) that are used for this kind of studies:

- random peptide libraries (**RPL**), suitable for fine characterization of protein-protein interactions (epitope and paratope mapping, recognition of residues crucial for receptor-ligand interactions involved in autoimmunity), identification of novel auto-antigens and small therapeutically applicable peptides;
- antigen fragment libraries (**AFL**), suitable for localization of epitopes;
- **cDNA libraries,** appropriate for discovery and identification of new antigens that bind disease-related antibodies and new specific therapeutic agents.

2. 1. Structural Analysis of Disease Implicated Protein Molecules

2. 1. 1. Epitope and Paratope Mapping Using Random Peptide Libraries

RPL display peptides of approximately 6–20 amino acids with randomized primary sequence, encoded by synthetic oligonucleotides. By screening RPL with the target (e.g. antibody, receptor) it is possible to either determine crucial contact residues of the already known ligand (antigen) or to identify potential new ligands (antigens) by performing homology searches of selected peptides against the protein databank. Papers covering the delineation of autoimmune epitopes with RPL are numerous, and the reader should refer to[10] for an overview. Our aim here is to present examples illustrating the usefulness of RPL for epitope mapping and the applicability of selected peptides for research and development of improved diagnostic reagents and novel therapeutics.

By screening RPL with monoclonal or polyclonal antibodies, linear, or more commonly, conformational epitopes on known antigens can be determined. The most straightforward is the determination of **linear epitopes**, as demonstrated by Blank et al., who screened hexapeptide RPL with monoclonal anti-$\beta_2$glycoprotein 1 antibodies (anti-β_2GP1), of a patient with antiphospholipid syndrome (**APS**), and selected three peptides with sequences resembling segments of β_2GP1 primary structure. When lengthened for 4–6 residues, peptides were able to inhibit anti-β_2GP1 induced *in vitro* endothelial cell activation and *in vivo* development of experimental APS in BALB/c mice, thus indicating the potential use of such peptides in targeted therapy.[11]

Often the selected motifs lack the homology with the auto-antigen, indicating these peptides mimic post-translationally modified epitopes or conformational epitopes, or recognize idiotypic instead of antigenic determinants of the auto-antibody.[12] Determination of post-translationally modified epitopes was recently undertaken by

van Bavel et al., who identified histone modifications implicated in auto-antibody formation in **SLE**. Selected sequences with the consensus motif IAAPAS/T corresponded to 27-KSAPAT-32 segment of histone H3, but contained neutral instead of cationic residue on position 27. The authors suggested apoptosis-induced post-translational modification of lysine 27, most likely methylation.[12] Determination of conformational epitopes is somewhat more stringent, since the RPL-derived motif has no homologue within the auto-antigen, and therefore sometimes requires additional techniques like molecular modelling and alanine mutagenesis. The latter involves systematic substitutions of deduced contact residues of antigen with alanine. Alanine removes the side chain beyond the β carbon and does not alter the backbone conformation. By evaluating the binding activities of the resulting array of mutant proteins the contribution to the binding energy made by the individual contact residue can be determined.[13] To characterize a **conformational epitope** region on glutamic acid decarboxylase, a major auto-antigen in **T1D**, heptapeptide RPL was screened with the human monoclonal antibody (b78). Residues comprising the selected motif were located on the surface of the homology model of glutamic acid decarboxylase C-terminus. Glutamic acid decarboxylase mutants constructed by substituting the implicated residues with alanine exhibited reduced reactivity with b78, thus confirming them as crucial contact residues.[14] In a study regarding **pemphigus vulgaris** the peptides, selected with monoclonal auto-antibodies, recognized idiotypic (V_H specific) instead of antigen binding determinants of the auto-antibody. These anti-idiotypic peptides were suggested to be used as adsorbents for depleting pathogenic auto-antibodies from patients' sera.[15] Balass et al. and Venkatesh et al. demonstrated potential therapeutic applicability of peptides mimicking conformational epitopes of myasthenia gravis (**MG**) associated pathogenic auto-antibodies to muscle acetylcholine receptor. Namely, the selected hexapeptide and the cyclized form of the selected 15-mer peptide were able to prevent experimental autoimmune MG in animal models induced by passive transfer of auto-antibodies.[16,17] Sometimes selected peptide sequences can not be linked to a specific auto-antigen, however that does not diminish their applicability. An example is the LALPPLAPNHHH peptide selected with IgG from patients with **ankylosing spondylitis** which exhibited higher positive and negative predictive values compared to inflammatory markers and may therefore be a candidate for ankylosing spondylitis specific serum biomarkers.[18]

The RPL can also be used to identify functional mimics of non-protein antigens. Many peptide mimotopes of dsDNA were selected by panning RPL with monoclonal or polyclonal anti-dsDNA antibodies, a laboratory hallmark of **SLE**.[19–21] Peptide immunization of mice and rabbits induced anti-dsDNA and other spontaneous murine lupus associated antibodies, confirming that with the use of RPL true peptide mimics of dsDNA can be obtained and at the same time suggesting a proteinaceous antigen could be the true trigger of the anti-dsDNA response.[19,21] The similar reactivity of SLE patients' sera when applied to dsDNA and mimotopes presents the possibility of using RPL derived peptides as diagnostic reagents. The main advantage of epitope mimicking peptides over auto-antigen molecules (bearing multiple epitopes) as diagnostic reagents is minor cross-reactivity and thus greater specificity of the former. This advantage was, for example, successfully applied to differentiate **T1D** related insulin auto-antibodies from T1D unrelated insulin auto-antibodies and therapy induced insulin antibodies, which was to that point unachievable by the conventional radiobinding assay in which molecular insulin was applied.[22]

Recently, we employed RPL in order to characterize **paratopes** (antigen-binding sites) of various **anti-β_2GP1**. The latter were pathological, derived from a patient with **APS**, or *in vitro*-induced by electro-oxidation. Namely, Omersel et al. reported on oxidative alteration of natural antibodies as a possible mechanism for the development of autoimmune reactions, based on similar binding characteristics of pathological and oxidatively altered healthy donors' IgGs revealed in *in vitro* tests.[23] To select peptides mimicking anti-β_2GP1 paratopes, we reversed the classical epitope mapping system. Here, RPL were screened over the auto-antigen (β_2GP1) and the bound phages were competitively eluted (displaced) by polyclonal anti-β_2GP1. Motif K-V/L-W-T/S-I/L-P-X, presenting putative pathologic anti-β_2GP1 paratopes, resembled the K-V/L-W-Q/T-I-P-X motif, presenting paratopes of oxidized IgGs. These motifs, in which the majority of amino acid residues denoted with X are positively charged or contain hydroxyl group, indicate the importance of electrostatic and hydrogen bonds in the β_2GP1- anti-β_2GP1 interaction, as previously suggested.[24]

2. 1. 2. Localization of Epitopes Using Antigen Fragment Libraries

Unlike the widely applicable RPL, **AFL** which display randomly generated fragments of a specific protein can only be used to localize epitopes to specific areas on that precise protein. These libraries are constructed for each protein separately by inserting digested cDNA (~100–300 bp) into a phagemid vector. Screening of AFL displaying fragments of known auto-antigens with monoclonal or polyclonal auto-antibodies is an effective way to identify linear protein epitopes.[25] Determination of conformational or discontinuous epitopes however is less probable. The attempt to identify an epitope of the antibody reported to recognize conformational epitopes on transglutaminase 2 (auto-antigen in celiac disease (**CD**)) failed, probably because the crucial residues on the displayed protein fragment could not recreate the same conformation as in the native protein.[26] Work by Blüthner et al.

provides an early example of the usefulness of an AFL for localization of linear epitopes on a protein with available cDNA. Screening of random fragments from the auto-antigen of polymiositis/scleroderma overlap syndrome (**PM/Scl-100**) with affinity purified anti-PM/Scl-100 antibodies narrowed down a major epitope area (157 amino acid residues) recognized by patients' sera to 23 consecutive amino acids and another minor epitope region (46 amino acid residues) to 21 amino acids.[27] In **primary biliary cirrhosis** two epitopic regions on disease associated nuclear auto-antigen sp 100 were determined by screening sp100 AFL with patients' antibodies.[28] The same strategy was also successfully applied in **vitiligo,** a chronic disorder causing skin depigmentation and in **autoimmune polyglandular syndrome type 1,** a disease that is characterized in part by hypoparathyroidism involving hypocalcemia, hyperphosphatemia, and low serum levels of parathyroid hormone. On the vitiligo associated melanin-concentrating hormone receptor 1 four epitopes were determined, including one targeted by antibodies that block the function of the receptor. On the calcium-sensing receptor, the auto-antigen related to autoimmune polyglandular syndrome type 1, one major and two minor epitopes located in the N-terminal of the receptor's extracellular domain were identified.[29,30] In addition to providing insight into the heterogeneity of the humoral immune response against an individual auto-antigen, AFL selection over patients' sera also allows determination of disease-specific markers present on the same auto-antigen. These markers can either be used to discriminate between different diseases with common auto-antigen specificity, as for example two recently identified epitopes of anti-topoisomerase I antibodies: one potentially characteristic for a subset of patients with **diffuse cutaneous systemic sclerosis** and the other for a subset of patients with **SLE**,[31] or to distinguish disease associated epitopes from disease unassociated epitopes, as was determined for anti-tyrosine-phosphatase-like protein antibodies implicated in **T1D**.[32]

2. 1. 3. Study of Receptor-Ligand Interactions Involved in Autoimmunity Using Random Peptide Libraries

It is well recognized that there is a genetic association between some autoimmune diseases and the expression of certain major histocompatibility complex (MHC) molecules whose function in autoimmunity involves the presentation of short peptides from auto-antigens to the nearby immune cells, and therefore their subsequent activation. Peptide structures recognized by individual disease associated MHC molecules, for example by **SLE** associated HLA DR-9 and **T1D** associated MHC II I-A^{g7}, have been identified with RPL. Based on selected motifs, the fragments of disease-responsible auto-antigens and specific T-cell clones can be identified which can further enlighten the mechanism of MHC-associated disease susceptibility.[33,34]

2. 2. Identification of New Auto-Antigens by Random Peptide Libraries and cDNA Libraries

Screening of RPL or phage cDNA libraries with patients' auto-antibodies has been proven to be extremely useful for the identification of novel, previously unknown auto-antigens. Candidates for new auto-antigens can be identified by performing a homology search of the short peptide sequences obtained by screening RPL with patients' IgGs against protein databases. Peptides reactive with the majority of **Sjögren's syndrome** sera exhibited sequence homology with tear lipocalin, αa-fodrin and Epstein-Barr virus-derived protein. While the first was confirmed as a new auto-antigen, the last implied the involvement of an Epstein-Barr virus infection in the pathogenesis of Sjögren's syndrome.[35] Osteopontin and importin beta, auto-antigens in **T1D,** were also identified by applying RPL. However, instead of a homology search, here the selected peptides were used to immunize the rabbits, and then the elicited rabbit antibodies were used to screen standard (λt11) human islets' cDNA library.[36] By this kind of multiplex method which involves a transfer of tissue or cell line derived cDNA library onto a membrane, immuno-detection of potential auto-antigens by patients' sera and working the way back to the encoding cDNA, many auto-antigens have been identified (reviewed in[37]).

The replacement of the standard λt11 cDNA library with the phage cDNA displayed library simplifies the multiplex identification of auto-antigens in terms of time and effort. Namely, in phage libraries characteristic proteins of the disease affected tissue (or of the corresponding cell lines) are displayed on the surface of phage particles and not in the cytoplasm of the host cell as in standard λt11 cDNA libraries. Therefore, the selection of serum-reactive proteins (auto-antigens) can be done by biopanning. With this approach, also called **serological antigen selection**, several disease specific auto-antigens, including the infrequent ones, can be determined simultaneously. An example is the recent detection of novel auto-antigens in **vitiligo**. By biopanning a phage cDNA library derived from cultured skin melanocytes over an IgG fraction of patients with vitiligo, a disease characteristic auto-antibody profile targeting gamma-enolase, alpha-enolase, heat-shock protein 90, osteopontin, ubiquitin-conjugating enzyme, translation-initiation factor 2, GTP binding protein Rab38 and melanin-concentrating hormone receptor 1 was identified.[38,39] Candidate auto-antigens for other autoimmune diseases identified by **serological antigen selection** are presented in Table 1. Along with clues regarding disease pathogenesis, these panels of auto-antigens characteristic for specific disease states present a valuable source for novel, more sensitive diagnostic and prediction

Table 1: Auto-Antigens Identified by Serological Antigen Selection

Autoimmune disease	Source of cDNA	Auto-Antigen
Atherosclerosis	ruptured peripheral human atherosclerotic plaques [40]	NKG2E, antigen E12 [40]
Multiple sclerosis	human MS brain plaques [41,42]	sperm associated antigen 16 [41] ERK5 [41]
Rheumatoid arthritis	human synovial sarcoma cell line SW982 [43] fibroblasts [44] RA synovial pannus tissue [45]	Sorcin, IGFBP-4 [43] ferritin heavy chain [44] minichromosome maintenance complex component 2, ribosomal protein S6, UH-RA.21 [45]
Systemic lupus erythematosus	fibroblasts [46]	ribosomal protein S20, ribosomal protein S13, ubiquitin-like protein PIC1, transcription factor-like protein MRG15 [46]
Autoimmune uveitis	human eyes [47] mice eyes [48]	tribbles homolog 2 [47] heterogeneous nuclear ribonucleoprotein H3 [48]
Vitiligo	cultured skin melanocytes [38, 39]	gamma-enolase, alpha-enolase, heat-shock protein 90, osteopontin, ubiquitin-conjugating enzyme, translation-initiation factor 2, GTP binding protein Rab38 [38] melanin-concentrating hormone receptor 1 [39]

markers, for example recently identified biomarkers for the non-invasive diagnosis of ruptured peripheral **atherosclerotic lesions**.[40]

2. 3. Peptide and Protein Libraries as a Source of Therapeutic Agents

The variety of severe side effects associated with non-specific established treatments (e.g. general immunosuppression) has prompted the development of biologic therapeutics targeting specific molecules that are involved in the pathogenesis of autoimmune diseases. Such potential biologic therapeutics are either focused on "pathogenic" cytokines and receptors involved in the inflammation process or in B- or T-cell proliferation, differentiation and maturation or on disease characteristic tissue changes. The development of these peptide/protein modulators and markers has been greatly facilitated by phage display technology.

2. 3. 1. Peptide and Protein Libraries for Therapeutic Targeting of Receptor-Ligand Interactions

Therapeutic targeting of cell surface molecules and soluble mediators, relevant to the function of immune cells, with human monoclonal antibodies is rapidly improving the treatment of autoimmune diseases. In the study of Grönwall et al., the **Affibody®**, a smaller alternative to antibodies, was used for targeting a **CD25**, T-cell activation regulating receptor over-expressed on cells involved in different inflammatory autoimmune diseases.[49] Affibody® libraries are pools of phage displayed protein scaffolds with identical backbones (45 residues) and variable surface-binding properties (13 randomized residues).[50] As suggested by the authors, the selected CD25-binding Affibody® molecules could be used as prospective therapeutic and medical-imaging agents.[49] Small peptide inhibitors represent another potential alternative to the established antibodies and are especially attractive due to their oral bioavailability and lower production costs.[51] Later studies demonstrated the applicability of **RPL** in this respect and present some potential candidates for the immunotherapy. The **CD40–CD154** interaction promotes the auto-reactive cell response and contributes to the proinflammatory and procoagulant state in SLE. The anti-CD154 antibody mediated therapeutical inhibition of CD40–CD154 interaction reduced the auto-antibody production in SLE, but it simultaneously resulted in thromboembolic complications. Recently, an alternative in the form of cyclic heptapeptide was selected by screening RPL with the hCD154 antigen. This peptide prevented some CD40–CD154 mediated biological activities *in vitro*, including B-cell activation, without triggering thrombotic effects.[52] Other therapeutically interesting peptides obtained by RPL interfered either with the inflammatory component of disease, as for example in peptides inhibiting the binding of tumor necrosis factor α (**TNFα**) and **interleukin-8** (inflammatory cytokines) to their cognate receptors or with catabolism of pathogenic auto-antibodies.[53,54] The inhibitory peptide, selected on the **neonatal Fc receptor** which otherwise assures long half-lives of antibody molecules by binding them, obstructed the respective interaction and thus promoted the therapeutically beneficial *in vivo* clearance of pathogenic antibodies.[55] In addition to being used as inhibitors, RPL derived peptides can also be applied to induce an active therapeutic B-cell response, as demonstrated by Perosa et al. They selected the **Rituximab** mimotope and used it to induce an immune response against the B-cell antigen CD20 in mice. Ho-

wever, the therapeutical applicability and suitability of this approach to replace passive immunotherapy with Rituximab in humans remains to be ascertained.[56]

Soluble domains of cell surface molecules or mutant variants of immune mediators constructed by phage display technology can also be applied for the therapeutical modulation of autoimmune response. An example of the first is the high-affinity **T-cell receptor** constructed by combining the phage display technology with protein engineering. A soluble form of T-cell receptor could inhibit the activation of auto-reactive T cells by directly blocking a specific peptide-MHC complex.[57] Screening of a library displaying mutant **TNF** variants, differing in six randomized amino acid residues, resulted in the isolation of TNF receptor 1- selective antagonist. Studies on animal models demonstrated that inhibition of the TNF receptor 1 mediated signalling by a respective TNF mutant could be effective in treating rheumatoid arthritis (RA).[58]

2. 3. 2. Random Peptide Libraries as a Source of Drug Delivery Agents

Screening of RPL over disease affected tissues represents an efficient way to isolate specific peptides that could be used as drug delivery systems or diagnostic and therapy monitoring markers. The isolation of tissue targeting peptides can either be performed *ex vivo*, as for example the selection of peptides targeting intestinal inflammatory mucosa that were selected on mouse isolated inflamed bowel,[59] or *in vivo* as in the selection of synovial microvascular endothelium homing peptides that were isolated on human microvascular endothelium transplanted into SCID mouse.[60] The potential therapeutical applicability of such peptides was best presented by Mi et al., who isolated the human synovia targeting peptide that facilitates uptake of therapeutic agents into human synovial cells. The peptide enabled the *in vivo* internalization of the proapoptotic agent which favourably resulted in apoptosis of hyperplastic synovia.[61] This course of therapy could be monitored by an *in vivo* reporter of apoptosis, such as one developed by Burtea et al. They used RPL derived phosphatidylserine-specific peptide to design the apoptosis detecting magnetic resonance imaging contrast agent and demonstrated its applicability for magnetic resonance imaging diagnosis of apoptosis-related pathologies by imaging **atherosclerotic lesions** in animal models.[62] Recently, another interesting application of phage display technology in the therapy of experimental autoimmune encephalomyelitis, animal model of **MS**, was described. The filamentous phages which upon nasal administration penetrate the central nervous system were used as carriers for immunodominant auto-epitope of myelin oligodendrocyte glycoprotein, displayed on their surface.[63] Namely, currently investigated antigen-specific therapeutical strategies for autoimmune diseases involve triggering unresponsiveness of auto-reactive T cells (self-tolerance) with high doses of auto-antigens.[64] Here, nasal administration of phage-myelin oligodendrocyte glycoprotein markedly improved clinical features in the MS animal model compared to untreated animals.[63]

3. Antibody Phage Display Libraries

Literature review indicates that phage display of human antibody fragments has had a large impact on the study of autoimmune diseases and substantially contributed to different aspects of that area. Phage antibody libraries present an improved alternative to hybridoma technology by allowing generation of fully human antibodies of miscellaneous specificities applicable in therapy or diagnostics.[65] Moreover they facilitate the isolation of human autoimmune antibodies' specificities which are difficult to obtain by immunization due to tolerance mechanisms. To be exact, antibody libraries are a subtype of cDNA libraries, prepared by cloning segments of human antibody genes into a phagemid vector. Usually, the antibody fragments are displayed in scFv (V_H and V_L joined by a short linker) or Fab (V_H-C_H and V_L-C_L) formats.[66]

Depending on the source of the Ig genes used for the cloning, 3 types of libraries are distinguished: 1. autoimmune (derived from B cells of a patient with autoimmune disease), 2. naive (derived from B cells of a healthy donor) and 3. synthetic libraries.[66]

The screening of autoimmune libraries over the corresponding auto-antigen usually results in isolation of specific high affinity antibody fragments; this is understandable given that in an immunized source V gene sequences encoding antigen binding sites/paratopes are enriched (Figure 1). On the other hand, naive and synthetic libraries contain antibody fragments of wider specificity that generally have medium or low affinities. Despite their given disadvantages, these libraries are commonly applied in the generation of antibodies for use in therapy or diagnostics which require high affinities. Inadequate affinity of selected fragments, along with pharmacological activity and pharmacokinetic profiles can be improved by *in vitro* affinity maturation.[66, 67] The latter includes introduction of random or targeted genetic changes into an antigen binding site/paratope, and screening of this secondary library of derivatives with altered binding activities for clones with improved affinity (Figure 1).

3. 1. Structural and Functional Analysis of Auto-Antibodies

Several studies have suggested that auto-antigen specific antibody fragments selected from autoimmune libraries adequately resemble the *in vivo* auto-antibody repertoire.[68]

Languren et al., who characterized phage derived antibody fragments of a patient with autoimmune throm-

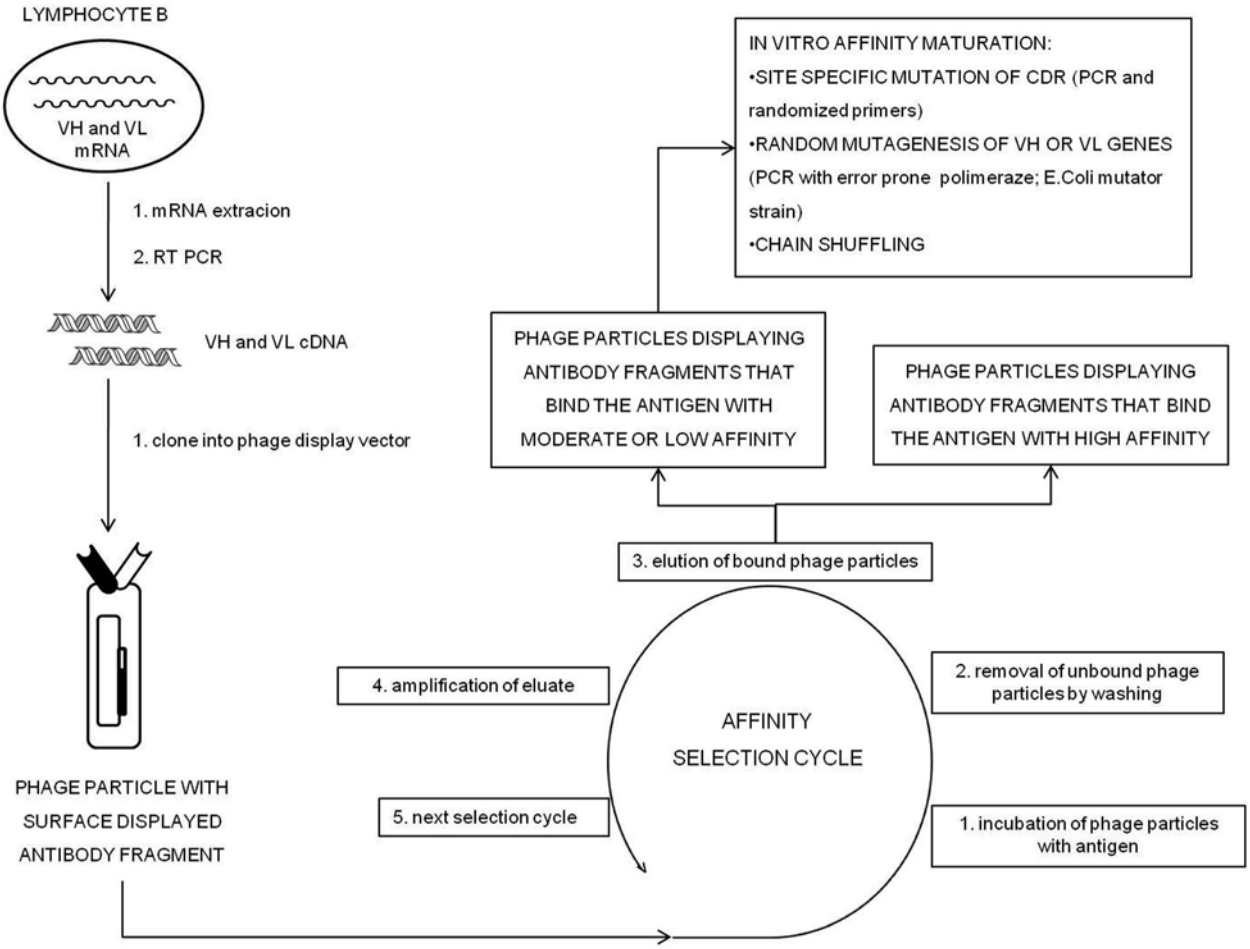

Figure 1: Generation of antibodies by phage display technology. Genes encoding heavy and light antibody chains are harvested from immune or naive B cells and cloned into a phagemid vector. The pool of antibody fragments displaying phages is submitted to sequential affinity selection cycles over an antigen of interest. The affinity of selected fragments is improved by *in vitro* maturation strategies.

bosis (in **APS**), noticed that they had a similar genetic origin as the previously reported APS-associated antibodies, studied with conventional techniques.[69]

In another study regarding epitope mapping of tissue transglutaminase (tTG), a **CD** associated auto-antigen, patient derived scFvs against the tTG were demonstrated to have identical specificity to the patient's serum antibodies against tTG.[70] The resemblance between specificities of serum and phage technology constructed auto-antibodies was also reported for **T1D, SLE, RA** and **thyroid autoimmunity** related auto-antibodies.[71–77]

Additionally, phage displayed antibody fragments possess pathological functions of naturally occurring auto-antibodies, as demonstrated for **pemphigus vulgaris and foliaceus** auto-antibody mediated skin blistering diseases. Namely, in animal model studies, the patient derived scFvs caused the same pathology as those observed in pemphigus patients.[78–80]

Therefore, by the structural (sequencing of selected V genes) analysis of phage displayed antibody fragments, information regarding the genetic restriction and clonality of B-cell response can be obtained. Specifically in **SLE** the analyses of phage derived high-affinity anti-dsDNA Fabs revealed usage of diverse V genes and their high level of homology to germ line, indicating that defects in central B-cell tolerance may contribute to pathogenesis of SLE. Furthermore, their binding to dsDNA was suggested to be dictated by basic-residue rich heavy chain complementarity-determining region 3 (H-CDR3) and V_L chain.[81–84] The H-CDR3 was also shown to be critical for pathogenicity of **pemphigus** associated auto-antibodies.[85] This kind of data together with binding (affinity, specificity) and functional characteristics of selected fragments can broaden our knowledge of different disease pathogenesis.

Antibody phage display libraries can also be applied to study the disease mechanisms directly, as demonstrated for **SLE**. Recognition that Fabs derived from a SLE patient target C1q complement component bound on early apoptotic cells, confirmed the link between SLE, apoptosis, and C1q. It has been hypothesized that SLE is associated with a defective clearance of apoptotic cells, which are

the source of auto-antigens targeted by various SLE associated antibodies.[86] C1q was suggested to promote clearance of apoptotic cells upon binding them, and the C1q deficiency observed in some patients could compromise that. A proximity selection of the scFv library on apoptotic cells confirmed the binding of C1q in the vicinity to the monocyte endocytic receptor CD91 and the surface calreticulin (a linker), both key molecules in the pathway of apoptotic recognition.[87]

In the last 10 years alone antibody phage display libraries have offered valuable information regarding the structure, specificity or function of auto-reactive responses, and thus have provided some insight into the pathogenesis of several autoimmune diseases. Structural analysis of phage displayed auto-antibodies specific for citrullinated proteins, derived from a **RA** patient, revealed the restricted usage of V genes (V_H3, V_H4, V1, and V1) and a high level of mutations in CDRs. As suggested by the authors, the usage of some uncommon V_H alleles which are not present in all individuals could indicate the genetic predisposition for the development of antibodies against citrullinated proteins.[71] Structural analysis of other RA-associated phage displayed antibodies, targeting glucose-6-phosphate isomerase or IgG's Fc region, also revealed extensive mutations in CDRs. A high level of somatic mutations in CDRs, assessed by comparing the sequence of clone and the closest germline sequence, indicates an affinity-matured antigen driven response.[71,76,88,89] Extensive mutations were also reported for auto-antibodies accompanying **autoimmune thrombocytopenic purpura**, an autoimmune hematologic disorder, and **SLE**.[90–94] In one of the studies these antibodies were reported to exhibit restrictive usage of V_H3 heavy chain genes, a subfamily that was also predominantly used in the **CD** associated anti-gliadin antibodies and in **Hepatitis C virus** associated auto-reactive cryoglobulins, targeting anti-Hepatitis C virus antibodies.[93,95,96]

Studies of **CD** immune response showed that phage display technology allows rather easy determination of the site of auto-antibody synthesis. Panning of phage antibody libraries from the peripheral and intestinal lymphocytes of CD patients over two major CD antigens (gliadin and tTG) revealed that the humoral response against tTG occurs only at the intestinal level, whereas that against gliadin occurs both peripherally and centrally.[97] It was also reported that V_H usage of intestine produced anti-tTG antibodies differs among serum positive and serum negative **T1D** patients.[98] A study where the introduction of phage displayed anti-tTG into mice caused ataxia further indicates the implication of anti-tTG antibodies in the neurological dysfunctions associated with CD.[99]

Auto-reactive libraries can also be very helpful in studies dealing with ambiguous autoimmune response specificities. In thyroid autoimmunity the alternating screening of patients derived library over two diverse, disease characteristic antigens provided evidence against a previously presumed shared cross-reactive epitope.[100] In **primary biliary cirrhosis** the structural modification of auto-reactive Fab (against pyruvate dehydrogenase complex) encoding V_H genes revealed the somatic mutation mediated epitope shift. Namely, the conversion genes encoding the highly mutated Fab, targeting the inner lipoyl domain, to the germline caused the redirection of Fab specificity towards a di-domain construct encompassing the inner lipoyl domain.[101]

3. 2. Identification of New Auto-Antigens

The ability of phage displayed antibodies to imitate natural specificities also makes them suitable for characterization of previously defined epitopes and identification of novel epitopes or even auto-antigens. In this regard a new auto-antigen involved in neutrophil destruction in **Felty´s syndrome** (combination of RA, splenomegaly and neutropenia) was identified. Screening of the cDNA library derived from a myelomonocytic cell line with neutrophils reactive Fab resulted in the identification of eukaryotic elongation factor 1A-1, against which the serum response was demonstrated in 66% of patients with Felty's syndrome.[102]

3. 3. Development of Diagnostic and Therapeutic Agents

The auto-reactive antibody libraries can be further considered as a source of prospective diagnostic and therapeutic agents. In development of the anti-actin IgA assay for diagnosis of **CD**, Fabbro et al. used the scFv library from intestinal lymphocytes of a CD patient to select for an anti-actin standard control antibody.[103] Ditzel et al. used the scFv library derived from a patient with **Sjögren's syndrome**, an autoimmune disorder characterized by the destruction of exocrine glands, to isolate a prospective marker for immunohistochemical analysis of tissue biopsies.[104] In **MG** the fragments of pathogenic auto-antibodies were demonstrated to prevent the loss of functional acetylcholine receptors by blocking the pathogenic epitopes.[105–107] The pathological activity of auto-antibodies in **bullous pemphigoid**, a skin blistering disease, was similarly prevented by recombinant Fabs.[108] In **Graves' disease** a mouse derived antibody library was the source of a therapeutically applicable antibody antagonizing the thyroid-stimulating antibodies responsible for hyperthyroidism.[109] Auto-reactive antibody libraries can be further used for the selection of anti-idiotypic antibodies, also capable of neutralizing pathogenic auto-antibodies. Reichlin et al. isolated such therapeutically applicable antibody targeting **SLE** accompanying cytopathic auto-antibody against ribosomal protein P. This anti-idiotypic agent inhibited immunoassay activity of antibodies against ribosomal protein P and their binding to the cells.[110]

Antibody phage display technology can also be exploited for the production of human antibodies targeting

specific, non-antigenic and otherwise naturally occurring molecules involved in the pathogenesis of autoimmune diseases. There are two procedures that apply phage display of antibody fragments to create fully human, high affinity antibodies:

a. The first includes the affinity selection of fully human, high-affinity antibody fragments (by combining antibody libraries and *in vitro* affinity maturation) which can be reformatted into IgG molecules and expressed in mammalian cell lines.[65]

b. The second approach is phage display driven humanization (guided selection), a method where the rodent antibody against a target of interest is gradually converted to human antibody with similar binding characteristics, as depicted in Figure 2.

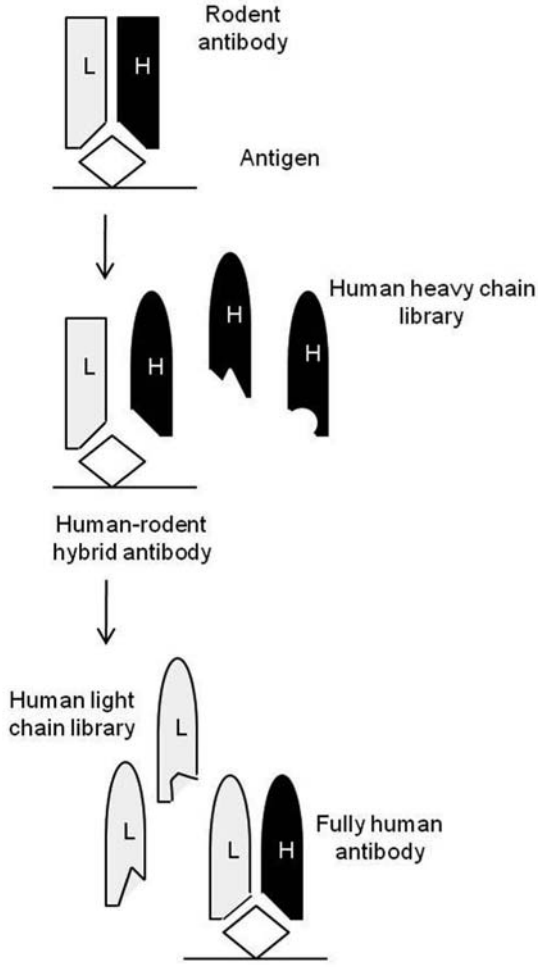

Figure 2: Humanization of the rodent antibody by phage display driven guided selection. By pairing the rodent V_L chain with the human V_H library, a human-rodent hybrid antibody library is obtained. After screening over the antigen of interest, the human V_H chain of the selected hybrid is paired with the human V_L library. The fully human library is then again screened over the antigen of interest and the human antibody with specificity corresponding to the starting rodent antibody is selected.

Adalimumab, the inhibitor of **TNFα** used for treatment of RA, clearly demonstrates the utility of phage display technology for development of therapeutic antibodies. Namely, this antibody was developed by guided selection and is the first fully human antibody drug approved for marketing.[111]

The same method has also been employed for the development of antibodies against **granulocyte macrophage colony stimulating factor**, **CD137** and **NKG2D receptor**, all potentially usable in the treatment of autoimmune diseases.[112–114] Neutralization of granulocyte macrophage colony stimulating factor is beneficial because this pro-inflammatory cytokine is aberrantly produced in RA and SLE, and it is involved in the development of organ-related autoimmune inflammatory diseases.[112,115] **CD137** is an inducible co-stimulatory receptor that promotes the survival and expansion of activated T cells. Antagonizing antibodies against CD137 therefore represent potential therapeutic agents for the reduction of T-cell mediated autoimmune diseases.[113] The anti-**NKGD2** antibody antagonizes an activating receptor expressed on natural killer and cytotoxic CD8+ T cells whose dysregulation may trigger or exacerbate autoimmune disorders like RA, MS, CD and T1D.[114,116,117] However, this is not the first report of therapeutically applicable NKG2D antagonizing human antibody. One year earlier, Kwong et al. selected NKG2D binding Fab from a naive human library and optimized its affinity towards NKG2D by chain shuffling. The respective Fab expressed in IgG1 format exhibited subnanomolar avidity towards NKG2D.[118] In addition to offering a broad range of specificities and multiple reapplications of a single library, this (first) approach is especially useful when auto-antigen-specific B-cell hybridomas are difficult to obtain. For example, hybridoma producing antibodies against **interleukin-6** is rather difficult to isolate, since interleukin-6 is used as a growth factor for B-cell hybridoma. However, screening of a human scFv library successfully resulted in the isolation of human anti-interleukin-6 antibody, potentially applicable in treatment of RA.[119] Furthermore, scFv or Fab displaying libraries are good starting points for the construction of **bispecific antibodies**, as demonstrated in a study by Marby et al. They separately selected two scFv fragments, one targeting **interleukin-17** and other targeting **interleukin-23**, and assembled them into a bispecific antibody. The resulting entity interrupts inflammatory pathways at two points and so exhibits enhanced therapeutic efficacy for inflammatory/autoimmune diseases.[120] However, phage display and affinity maturation technology also produced many monospecific antibodies or antibody fragments that show a potential for therapeutic use. These either interrupt disease accompanying inflammatory reactions (e.g. antibodies against **monocyte chemotactic protein-1**) or downregulate proliferation, and the differentiation and activation of auto-reactive immune cells (e.g. antibodies against **interleukin-21 receptor**,[67] **CD40 recep-**

tor,[121] **B7RP-1 costimulatory molecule** and **MHC-peptide complex**).[122–124] The most promising among phage library derived antibodies is **Belimumab**, a human antibody that selectively depletes B cells through inhibition of a B-lymphocyte stimulator. Belimumab has demonstrated considerable clinical benefit in SLE in terms of reduced disease activity and flare rates and is currently under FDA review for marketing approval.[125]

Antibody fragments specifically targeting disease affected tissues without directly affecting the precise molecule (receptor or mediator) can also be considered therapeutically applicable in terms of targeted therapy. One example is scFv specific to damaged cartilage in **RA** which was selected by screening a semi-synthetic phage display human antibody library over reactive oxygen species-modified collagen type II. The reduced inflammation in arthritic mice, achieved by fusing the selected scFv with TNFα depleting protein, confirmed that the respective scFv can be used as a drug-delivery capable of targeting arthritic joints.[126]

4. Conclusion

The study of protein-protein interactions (e.g. autoantibody – auto-antigen) is important for understanding the pathogenesis of autoimmune diseases and feasible therapy. The vast number of papers where phage display has been used in autoimmunity research clearly verifies the power of this technology. It lies in its simplicity, reliability, throughput and the ability to identify disease specific mimotopes, despite the unknown etiologic agent. Therapy for autoimmune diseases has particularly benefited from phage display technology and its harvesting is long from being completed.

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6. References

1. P. Marrack, J. Kappler, B. L. Kotzin, *Nat Med.* **2001**, *7*, 899–905.
2. N. R. Rose, I. R. Mackay, in: N. R. Rose, I. R. Mackay (Ed.): The Autoimmune Diseases, Academic Press, San Diego, CA, USA, **2006,** pp. *xix–xxv.*
3. M. A. Hemminga, W. L. Vos, P. V. Nazarov, R. B. Koehorst, C. J. Wolfs, R. B. Spruijt, D. Stopar, *Eur Biophys J.* **2010**, *39*, 541–550.
4. M. Russel, H. B. Lowman, T. Clackson, in: T. Clackson, H. B. Lowman (Ed.): Phage display. A practical approach, Oxford University Press, New York, NY, USA, **2004**, pp. *1–26.*
5. G. P. Smith, *Science.* **1985**, *228*, 1315–1317.
6. T. Bratkovic, *Cell Mol Life Sci.* **2010**, *67*, 749–767.
7. J. L. Jestin, *Biochimie.* **2008**, *90*, 1273–1278.
8. J. Pande, M. M. Szewczyk, A. K. Grover, *Biotechnol Adv.* **2010**, *28*, 849–858.
9. M. Lunder, T. Bratkovic, G. Anderluh, B. Strukelj, S. Kreft, *Acta Chim Slov*. **2008**, *55*, 233–235.
10. Z. Jadali, M. H. Sanati, *Iran J Allergy Asthma Immunol.* **2008**, *7*, 115–131.
11. M. Blank, Y. Shoenfeld, S. Cabilly, Y. Heldman, M. Fridkin, E. Katchalski-Katzir, *Proc Natl Acad Sci USA*. **1999**, *96*, 5164–5168.
12. C. C. van Bavel, J. W. Dieker, Y. Kroeze, W. P. Tamboer, R. Voll, S. Muller, J. H. Berden, J. van der Vlag, *Ann Rheum Dis.* **2011**, *70*, 201–207.
13. K. L. Morrison, G. A. Weiss, *Curr Opin Chem Biol*. **2001,** *5*, 302–307.
14. K. H. O'Connor, J. P. Banga, C. Darmanin, O. El-Kabbani, I. R. Mackay, M. J. Rowley, *J Autoimmun*. **2006**, *26*, 172–181.
15. A. S. Payne, D. L. Siegel, J. R. Stanley, *J Invest Dermatol.* **2007**, *127*, 1681–1691.
16. M. Balass, Y. Heldman, S. Cabilly, D. Givol, E. Katchalski-Katzir, S. Fuchs, *Proc Natl Acad Sci U S A.* **1993**, *90*, 10638–10642.
17. N. Venkatesh, S. H. Im, M. Balass, S. Fuchs, E. Katchalski-Katzir, *Proc Natl Acad Sci U S A.* **2000**, *97*, 761–766.
18. M. Wang, X. Li, J. Chen, Y. Zhou, H. Cao, X. Wu, H. Jiang, *Rheumatol Int.* **2010**.
19. A. Sharma, D. Isenberg, B. Diamond, *Rheumatology.* **2003**, *42*, 453–460.
20. W. Zhang, M. Reichlin, *Clin Immunol.* **2005**, *117*, 214–220.
21. Y. Sun, K. Y. Fong, M. C. Chung, Z. J. Yao, *Int Immunol*. **2001**, *13*, 223–232.
22. B. Franke, T. S. Galloway, T. J. Wilkin, *Diabetes Metab Res Rev.* **2005**, *21*, 395–415.
23. J. Omersel, I. Jurgec, S. Cucnik, T. Kveder, B. Rozman, S. Sodin-Semrl, B. Bozic, *Autoimmun Rev*. **2008**, *7*, 523–529.
24. I. Jurgec, M. Lunder, T. Bratkovic, S. Cucnik, T. Kveder, B. Rozman, B. Bozic, *Ann Rheum Dis*. **2009**, *68(Suppl I)*, A4.
25. M. B. Irving, O. Pan, J. K. Scott, *Curr Opin Chem Biol.* **2001**, *5*, 314–324.
26. R. Di Niro, F. Ferrara, T. Not, A. R. Bradbury, F. Chirdo, R. Marzari, D. Sblattero, *Biochem J.* **2005**, *388*, 889–894.
27. M. Blüthner, E. K. Bautz, F. A. Bautz, *J Immunol Methods.* **1996**, *198*, 187–198.
28. M. Blüthner, C. Schäfer, C. Schneider, F.A. Bautz, *Autoimmunity.* **1999**, *29*, 33–42.
29. N. G. Gavalas, R. V. Gottumukkala, D. J. Gawkrodger, P. F. Watson, A. P. Weetman, E. H. Kemp, *Exp Dermatol*. **2009**, *18*, 454–463.
30. E. H. Kemp, N. G. Gavalas, S. Akhtar, K. J. Krohn, J. C. Pallais, E. M. Brown, P. F. Watson, A. P. Weetman, *J Bone Miner Res.* **2010**, *25*, 132–140.
31. D. Simon, T. Czömpöly, T. Berki, T. Minier, A. Peti, E. Tóth,

L. Czirják, P. Németh, *Int Immunol.* **2009**, *21*, 415–422.
32. D. Miao, L. Yu, C. Tiberti, D. D. Cuthbertson, M. Rewers, U. di Mario, G. S. Eisenbarth, F. Dotta, *J Autoimmun*. **2002**, *18*, 191–196.
33. S. Fujisao, S. Matsushita, T. Nishi, Y. Nishimura, *Hum Immunol*. **1996**, *45*, 131–136.
34. S. Gregori, E. Bono, F. Gallazzi, J. Hammer, L. C. Harrison, L. Adorini, *Int Immunol.* **2000**, *12*, 493–503.
35. R. Navone, C. Lunardi, R. Gerli, E. Tinazzi, D. Peterlana, C. Bason, R. Corrocher, A. Puccetti, *J Autoimmun*. **2005**, *25*, 229–234.
36. T. O. Ola, P. A. Biro, M. I. Hawa, J. Ludvigsson, M. Locatelli, M. A. Puglisi, G. F. Bottazzo, A. Fierabracci, *J Autoimmun*. **2006**, *26*, 197–207.
37. P. Krebs, M. Kurrer, U. Sahin, O. Türeci, B. Ludewig, *Autoimmun Rev.* **2003**, *2*, 339–345.
38. E. A. Waterman, D. J. Gawkrodger, P. F. Watson, A. P. Weetman, E. H. Kemp, *J Invest Dermatol.* **2010**, *130*, 230–240.
39. E. H. Kemp, E. A. Waterman, B. E. Hawes, K. O'Neill, R. V. S. R. K. Gottumukkala, D. J. Gawkrodger, A. P. Weetman, P. F. Watson, *J Clin Invest.* **2002**, *109*, 923–930.
40. K. B. Cleutjens, B. C. Faber, M. Rousch, R. van Doorn, T. M. Hackeng, C. Vink, P. Geusens, H. ten Cate, J. Waltenberger, V. Tchaikovski, M. Lobbes, V. Somers, A. Sijbers, D. Black, P. J. Kitslaar, M. J. Daemen, *J Clin Invest*. **2008**, *118*, 2979–2985.
41. V. Somers, C. Govarts, K. Somers, R. Hupperts, R. Medaer, P. Stinissen, *J Immunol.* **2008**, *180*, 3957–3963.
42. C. Govarts, K. Somers, R. Hupperts, P. Stinissen, V. Somers, *Autoimmunity.* **2009**, *42*, 699–704.
43. M. Niwa, K. Fukuoka, T. Fujimoto, I. N. Maruyama, *J Biotechnol*. **2004**, *114*, 55–58.
44. D. Mewar, D. J. Moore, S. Young-Min, M. L. Bertolaccini, M. A. Khamashta, P. F. Watson, A. G. Wilson, *Arthritis Rheum.* **2005**, *52*, 3868–3872.
45. K. Somers, P. Geusens, D. Elewaut, F. De Keyser, J. L. Rummens, M. Coenen, M. Blom, P. Stinissen, V. Somers, *J Autoimmun.* **2011**, *36*, 33–46.
46. E. H. Kemp, L. M. Herd, E. A. Waterman, A. G. Wilson, A. P. Weetman, P. P. Watson, *Biochem Biophys Res Commun.* **2002**, *298*, 169–177.
47. Y. Zhang, J. L. Davis, W. Li, *Mol Immunol.* **2005**, *42*, 1275–1281.
48. Y. Kim, N. B. Caberoy, G. Alvarado, J. L. Davis, W. J. Feuer, W. Li, *Clin Immunol.* **2011,** *138,* 60–66.
49. C. Grönwall, E. Snelders, A. J. Palm, F. Eriksson, N. Herne, S. Ståhl, *Biotechnol Appl Biochem.* **2008**, *50*, 97–112.
50. M. Friedman, E. Nordberg, I. Höidén-Guthenberg, H. Brismar, G. P. Adams, F. Y. Nilsson, J. Carlsson, S. Ståhl, *Protein Eng Des Sel.* **2007**, *20*, 189–199.
51. E. W. St Clair, *Curr Opin Immunol*. **2009**, *21*, 648–657.
52. I. Deambrosis, S. Lamorte, F. Giaretta, L. Tei, L. Biancone, B. Bussolati, G. Camussi, *J Mol Med*. **2009**, *87*, 181–197.
53. J. Zhang, L. Zheng, A. Zhao, B. Gao, N. L. Liu, F. Wang, J. Dong, Z. T. Xin, N. S. Shao, H. X. Wang, Y. N. Xue, *Biochem Biophys Res Commun.* **2003**, *310*, 1181–1187.
54. M. Houimel, L. Mazzucchelli, *J Leukoc Biol.* **2009**, *85*, 728–738.
55. A. R. Mezo, K. A. McDonnell, C. A. Hehir, S. C. Low, V. J. Palombella, J. M. Stattel, G. D. Kamphaus, C. Fraley, Y. Zhang, J. A. Dumont, A. J. Bitonti, *Proc Natl Acad Sci USA.* **2008**, *105*, 2337–2342.
56. F. Perosa, E. Favoino, C. Vicenti, A. Guarnera, V. Racanelli, V. De Pinto, F. Dammacco, *J Immunol.* **2009**, *182*, 416–423.
57. J. M. Boulter, B. K. Jakobsen, *Clin Exp Immunol.* **2005**, *142*, 454–460.
58. T. Nomura, Y. Abe, H. Kamada, M. Inoue, T. Kawara, S. Arita, T. Furuya, K. Minowa, Y. Yoshioka, H. Shibata, H. Kayamuro, T. Yamashita, K. Nagano, T. Yoshikawa, Y. Mukai, S. Nakagawa, S. Tsunoda, Y. Tsutsumi, *Pharmazie*. **2010**, *65*, 93–96.
59. T. Takagi, T. Arisawa, K. Yamamoto, I. Hirata, H. Nakano, M. Sawada, *Clin Exp Pharmacol Physiol.* **2007**, *34*, 286–289.
60. L. Lee, C. Buckley, M. C. Blades, G. Panayi, A. J. George, C. Pitzalis, *Arthritis Rheum.* **2002**, *46*, 2109–2120.
61. Z. Mi, X. Lu, J. C. Mai, B. G. Ng, G. Wang, E. R. Lechman, S. C. Watkins, H. Rabinowich, P. D. Robbins, *Mol Ther.* **2003**, *8*, 295–305.
62. C. Burtea, S. Laurent, E. Lancelot, S. Ballet, O. Murariu, O. Rousseaux, M. Port, L. Vander Elst, C. Corot, R. N. Muller, *Mol Pharm*. **2009**, *6*, 1903–1919.
63. I. S. Rakover, N. Zabavnik, R. Kopel, M. Paz-Rozner, B. Solomon, *J Neuroimmunol*. **2010**, *225*, 68–76.
64. S. D. Miller, D. M. Turley, J. R. Podojil, *Nat Rev Immunol*. **2007**, *7*, 665–677.
65. J. Osbourn, L. Jermutus, A. Duncan, *Drug Discov Today.* **2003**, *8*, 845–851.
66. H. M. Azzazy, W. E. Highsmith Jr., *Clin Biochem.* **2002**, *35*, 425–445.
67. Y. Vugmeyster, H. Guay, P. Szklut, M. D. Qian, M. Jin, A. Widom, V. Spaulding, F. Bennett, L. Lowe, T. Andreyeva, D. Lowe, S. Lane, G. Thom, V. Valge-Archer, D. Gill, D. Young, L. Bloom, *MAbs.* **2010**, *2*, 335–346.
68. P. Pichurin, J. Guo , X. Yan , B. Rapoport , S. M. McLachlan, *Thyroid.* **2001**, *11*, 301–313.
69. M. Languren, B. Becerril, A. R. Cabral, L. E. Fernández-Altuna, V. Pascual, D. F. Hernández-Ramírez, J. Cabiedes, *J Autoimmun.* **2006**, *26*, 57–65.
70. D. Sblattero, F. Florian, E. Azzoni, T. Zyla, M. Park, V. Baldas, T. Not, A. Ventura, A. Bradbury, R. Marzari, *Eur J Biochem*. **2002**, *269*, 5175–5181.
71. J. M. Raats, E. M. Wijnen, G. J. Pruijn, F. H. van den Hoogen, W. J. van Venrooij, *J Rheumatol.* **2003**, *30*, 1696–1711.
72. K. Jury, P. Söhnlein, M. Vogel, W. Richter, *Diabetes*. **2001**, *50*, 1976–1982.
73. S. Zampieri, M. Mahler, M. Blüthner, Z. Qiu, K. Malmegrim, A. Ghirardello, A. Doria, W. J. van Venrooij, J. M. Raats, *Cell Mol Life Sci.* **2003**, *60*, 588–598.
74. I. Del Rincon, M. Zeidel, E. Rey, J. B. Harley, J. A. James, M. Fischbach, I. Sanz, *J Immunol.* **2000**, *165*, 7011–7016.

75. P. Pichurin, J. Guo, X. Yan, B. Rapoport, S. M. McLachlan, *Thyroid.* **2001**, *11*, 301–313.
76. M. Schaller, D. R. Burton, H. J. Ditzel, *Nat Immunol.* **2001**, *2*, 746–753.
77. P. N. Pichurin, J. Guo, V. Estienne, P. Carayon, J. Ruf, B. Rapoport, S. M. McLachlan, *Thyroid.* **2002**, *12*, 1085–1095.
78. A. S. Payne, K. Ishii, S. Kacir, C. Lin, H. Li, Y. Hanakawa, K. Tsunoda, M. Amagai, J. R. Stanley, D. L. Siegel, *J Clin Invest.* **2005**, *115*, 888–899.
79. K. Ishii, C. Lin, D. L. Siegel, J. R. Stanley, *J Invest Dermatol.* **2008**, *128*, 939–948.
80. J. Yamagami, S. Kacir, K. Ishii, A. S. Payne, D. L. Siegel, J. R. Stanley, J Immunol. **2009**, *183*, 5615–5621.
81. P. Roben, S. M Barbas, L. Sandoval, J. M. Lecerf, B. D. Stollar, A. Solomon, G. J. Silverman, *J Clin Invest.* **1996,** *98*, 2827–2837.
82. S. M. Barbas, H. J. Ditzel, E. M. Salonen, W. P. Yang, G. J. Silverman, D. R. Burton, *Proc Natl Acad Sci USA.* **1995**, *92*, 2529–2533.
83. S. N. Seal, R. M. Hoet, J. M. Raats, M. Z. Radic, *Arthritis Rheum.* **2000**, *43*, 2132–2138.
84. M. Suzuki, H. Takemura, H. Suzuki, T. Sumida, *Biochem Biophys Res Commun.* **2000**, *271*, 240–243.
85. J. Yamagami, A. S. Payne, S. Kacir, K. Ishii, D. L. Siegel, J. R. Stanley, *J Clin Invest.* **2010,** *120*, 4111–4117.
86. C. Bigler, M. Schaller, I. Perahud, M. Osthoff, M. Trendelenburg, *J Immunol*. **2009**, *183*, 3512–3521.
87. S. Donnelly, W. Roake, S. Brown, P. Young, H. Naik, P. Wordsworth, D. A. Isenberg, K. B. Reid, P. Eggleton, *Arthritis Rheum*. **2006**, *54*, 1543–1556.
88. W. J. Van Esch, C. C. Reparon-Schuijt, H. J. Hamstra, C. Van Kooten, T. Logtenberg, F. C. Breedveld, C. L. Verweij, *Clin Exp Immunol.* **2003**, *131*, 364–376.
89. W. J. Van Esch, C. C. Reparon-Schuijt, H. J. Hamstra, C. Van Kooten, T. Logtenberg, F. C. Breedveld, C. L. Verweij, *J Autoimmun.* **2002**, *19*, 241–250.
90. M. J. Jacobin, R. Robert, O. Pouns, J. Laroche-Traineau, A. Nurden, K. Peter, M. Little, G. Clofent-Sanchez, *Clin Immunol.* **2003**, *108*, 199–210.
91. M. J. Jacobin, J. Laroche-Traineau, M. Little, A. Keller, K. Peter, M. Welschof, A. Nurden, G. Clofent-Sanchez, *J Immunol*. **2002**, *168*, 2035–2045.
92. M. Hamidpour, M. Behrendt, B. Griffiths, L. Partridge, N. Lindsey, *Eur J Haematol.* **2006**, *76*, 331–338.
93. J. H. Roark, J. B. Bussel, D. B. Cines, D. L. Siegel, *Blood.* **2002**, *100*, 1388–1398.
94. M. Schaller, C. Bigler, D. Danner, H. J. Ditzel, M. Trendelenburg, *J Immunol.* **2009**, *183*, 8225–8231.
95. C. Rhyner, M. Weichel, P. Hübner, G. Achatz, K. Blaser, R. Crameri, *Immunology* **2003**, *110*, 269–274.
96. M. Perotti, N. Ghidoli, R. Altara, R. A. Diotti, N. Clementi, D. De Marco, M. Sassi, M. Clementi, R. Burioni, N. Mancini, *Autoimmun Rev.* **2008**, *7*, 468–472.
97. R. Marzari, D. Sblattero, F. Florian, E. Tongiorgi, T. Not, A. Tommasini, A. Ventura, A. Bradbury, *J Immunol.* **2001**, *166*, 4170–4176.
98. M. Maglio, F. Florian, M. Vecchiet, R. Auricchio, F. Paparo, R. Spadaro, D. Zanzi, L. Rapacciuolo, A. Franzese, D. Sblattero, R. Marzari, R. Troncone, *Diabetes.* **2009**, *58*, 1578–1584.
99. S. Boscolo, A. Lorenzon, D. Sblattero, F. Florian, M. Stebel, R. Marzari, T. Not, D. Aeschlimann, A. Ventura, M. Hadjivassiliou, E. Tongiorgi, *PLoS One.* **2010**, *5*, e9698.
100. F. Latrofa, P. Pichurin, J. Guo, B. Rapoport, S. M. McLachlan, *J Clin Endocrinol Metab*. **2003**, *88*, 371–378.
101. K. N. Potter, R. K. Thomson, A. Hamblin, S. D. Richards, J. G. Lindsay, F. K. Stevenson, *J Mol Biol.* **2001**, *306*, 37–46.
102. H. J. Ditzel, Y. Masaki, H. Nielsen, L. Farnaes, D. R Burton, *Proc Natl Acad Sci USA.* **2000**, *97*, 9234–9239.
103. E. Fabbro, L. Rubert, S. Quaglia, F. Ferrara, V. Kiren, A. Ventura, T. Not, *Clin Chim Acta.* **2008**, *390*, 134–137.
104. T. Maruyama, I. Saito, Y. Hayashi, E. Kompfner, R. I. Fox, D. R. Burton, H. J. Ditzel, *Am J Pathol.* **2004**, *165*, 53–61.
105. E. Rey, M. Zeidel, C. Rhine, J. Tami, K. Krolick, M. Fischbach, I. Sanz, *Clin Immunol.* **2000**, *96*, 269–279.
106. E. Fostieri, S. J. Tzartos, S. Berrih-Aknin, D. Beeson, A. Mamalaki, *Eur J Immunol.* **2005**, *35*, 632–643.
107. F. Meng, M. H. Stassen, S. Schillberg, R. Fischer, M. H. De Baets, *Autoimmunity*. **2002**, *35*, 125–133.
108. G. Wang, H. Ujiie, A. Shibaki, W. Nishie, Y. Tateishi, K. Kikuchi, Q. Li, J. R. McMillan, H. Morioka, D. Sawamura, H. Nakamura, H. Shimizu, *Am J Pathol.* **2010**, *176*, 914–925.
109. Y. Zhao, S. L. Wang, Q. Li, J. Ye, K. M. Chen, E. J. Tian, Z. P. Chen, *Hybridoma (Larchmt).* **2008**, *27*, 445–451.
110. W. Zhang, M. Reichlin, *Clin Immunol* **2005**, *114*, 130–136.
111. J. Osbourn, M. Groves, T. Vaughan, *Methods.* **2005**, *36*, 61–68.
112. E. M. Krinner, T. Raum, S. Petsch, S. Bruckmaier, I. Schuster, L. Petersen, R. Cierpka, D. Abebe, M. Mølhøj, A. Wolf, P. Sørensen, M. Locher, P. A. Baeuerle, J. Hepp, *Mol Immunol*. **2007**, *44*, 916–925.
113. U. H. Lee, J. H. Son, J. J. Lee, B. Kwon, J. W. Park, B. Se Kwon B, *J Immunother.* **2004**, *27*, 201–210.
114. J. Steigerwald, T. Raum, S. Pflanz, R. Cierpka, S. Mangold, D. Rau, P. Hoffmann, M. Kvesic, C. Zube, S. Linnerbauer, J. Lumsden, M. Sriskandarajah, P. Kufer, P. A. Baeuerle, J. Volkland, *MAbs.* **2009**, *1*, 115–127.
115. I. Sonderegger, G. Iezzi, R. Maier, N. Schmitz, M. Kurrer, M. Kopf, *J Exp Med.* **2008**, *205*, 2281–2294.
116. S. Caillat-Zucman, *Hum Immunol.* **2006**, *67*, 204–207.
117. K. Ogasawara, J. A. Hamerman, L. R. Ehrlich, H. Bour-Jordan, P. Santamaria, J. A. Bluestone, L. L. Lanier, *Immunity.* **2004**, *20*, 757–767.
118. K. Y. Kwong, S. Baskar, H. Zhang, C. L. Mackall, C. Rader, *J Mol Biol.* **2008**, *384*, 1143–1156.
119. R. Gejima, K. Tanaka, T. Nakashima, S. Hashiguchi, Y. Ito, K. Yoshizaki, K. Sugimura, *Hum Antibodies.* **2002**, *11*, 121–129.
120. R. Mabry, K. E. Lewis, M. Moore, P. A. McKernan, T. R. Bukowski, K. Bontadelli, T. Brender, S. Okada, K. Lum, J. West, J. L. Kuijper, D. Ardourel, S. Franke, L. Lockwood,

T. Vu, A. Frank, M. W. Appleby, A. Wolf, B. Reardon, N. B. Hamacher, B. Stevens, P. Lewis, K. B. Lewis, D. G. Gilbertson, M. Lantry, S. H. Julien, C. Ostrander, C. Chan, K. Byrnes-Blake, J. Brody, S. Presnell, B. Meengs, S. D. Levin, M. Snavely, *Protein Eng Des Sel.* **2010**, *23*, 115–127.

121. P. Ellmark, C. Ottosson, C. A. Borrebaeck, A. C. Malmborg Hager, C. Furebring, *Immunology.* **2002**, *106*, 456–463.
122. K. Yoshinaga, M. Matsumoto, M. Torikai, K. Sugyo, S. Kuroki, K. Nogami, R. Matsumoto, S. Hashiguchi, Y. Ito, T. Nakashima, K. Sugimura, *J Biochem.* **2008**, *143*, 593–601.
123. J. Engberg, M. Krogsgaard, L. Fugger, *Immunotechnology.* **1999**, *4*, 273–278.
124. M. Krogsgaard, K. W. Wucherpfennig, B. Cannella, B. E. Hansen, A. Svejgaard, J. Pyrdol, H. Ditzel, C. Raine, J. Engberg, L. Fugger, *J Exp Med.* **2000**, *191*, 1395–1412.
125. K. P. Baker, B. M. Edwards, S. H. Main, G. H. Choi, R. E. Wager, W. G. Halpern, P. B. Lappin, T. Riccobene, D. Abramian, L. Sekut, B. Sturm, C. Poortman, R. R. Minter, C. L. Dobson, E. Williams, S. Carmen, R. Smith, V. Roschke, D. M. Hilbert, T. J. Vaughan, V. R Albert, *Arthritis Rheum.* **2003**, *48*, 3253–3265.
126. C. Hughes, B. Faurholm, F. Dell'Accio, A. Manzo, M. Seed, N. Eltawil, A. Marrelli, D. Gould, C. Subang, A. Al-Kashi, C. De Bari, P. Winyard, Y. Chernajovsky, A. Nissim, *Arthritis Rheum.* **2010**, *62*, 1007–1016.

Povzetek

Avtoimunske bolezni, ki prizadanejo približno 3 % prebivalstva, predstavljajo resen zdravstveni problem in so eden izmed pomembnih vzrokov smrtnosti. Kot take so predmet številnih raziskav, katerih cilj je razumevanje patogenih mehanizmov posamezne bolezni ter razvoj učinkovitejših diagnostičnih testov in terapevtskih pristopov. Tehnologija bakteriofagnega prikaza je zelo uporabno orodje za sistematično prou Izdelana je bila evanje interakcij med proteini. Slednje so gonilna sila (pato)fizioloških procesov, tudi avtoimunskih. S komercialno dostopnimi bakteriofagnimi predstavitvenimi knjižnicami peptidov lahko odkrivamo in ovrednotimo vezavna mesta med protitelesi in antigeni ter receptorji in ligandi, s čimer dobimo povsem osnoven, biokemijski vpogled v proteinsko mrežo mehanizma bolezni. Bakteriofagne predstavitvene knjižnice proteinov, s katerimi lahko na površini bakteriofaga izrazimo celotne domene (avtoimunskih) protiteles, receptorjev ali ligandov omogočajo razvoj bioloških zdravil uporabnih v terapiji avtoimunskih bolezni. Prav tako so bakteriofagne predstavitvene knjižnice protiteles, ki so dober približek *in vivo* protitelesnemu odzivu občutno olajšale proučevanje strukture in funkcije avtoimunskih protiteles ter njihove vloge v patologiji bolezni. V preglednem članku smo sistematično predstavili raznovrstne možnosti uporabe in potencialne prednosti tehnologije bakteriofagnega prikaza pri raziskovanju avtoimunskih bolezni. Članek ponuja temeljit pregled študij, v katerih so s pomočjo tehnologije bakteriofagnega prikaza raziskovali patološke mehanizme avtoimunskih bolezni in/ali razvijali nova diagnostična orodja ter usmerjene terapevtske pristope.