REVIEW ARTICLE
Iran J Allergy Asthma Immunol
September 2008; 7(3): 115-131

The Autoimmune Diseases Manifested by Production of Autoantibodies: The Autoantigens Identified by Random Peptide Library

Zohreh Jadali[1] and Mohammad Hossein Sanati [2]

[1] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2] Clinical Genetics Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Received: 24 December 2007; Received in revised form: 16 March 2008; Accepted: 6 April 2008

ABSTRACT

Phage-displayed random peptide libraries (RPL) provide a powerful technique for identification, structural and functional analysis of ligands for many different target molecules, including, antibodies, receptors or other proteins. This strategy has been verified to be an effective tool for research in immunology and successfully has been used to determine the target sequence for monoclonal and polyclonal antibodies. The peptide library approach provides great promise for characterization of ligands with no prior information concerning antibody specificity. This would allow the recognition of candidate antigens involved in initiation or perpetuation of autoimmune diseases. This technology also offers the potential for new therapeutic opportunities, production of diagnostic reagents, or even development of effective new vaccines. This review focuses on studies regarding the identification of autoantigens recognized by antibodies in autoimmune diseases using phage-display peptide libraries.

Key words: Autoantigens; Autoimmune diseases; Random peptide library

INTRODUCTION

For the first time George P. Smith showed that exogenous peptides could be displayed on the surface of filamenthous bacteriophage. He also demonstrated that the recombinant phage could be affinity selected by a polyclonal antibody, specific for the peptide insert.[1] After a short time the first phage-display libraries were separately reported in three publications[2-4]. This technology has quickly developed into an efficient tool for the detection and characterization of various ligand-receptor interactions[5-7] and has been successfully employed for several applications including epitope mapping, vaccine development and identification of peptides mimics of nonpeptide ligands.[8,9] During the last years the search for specific high-affinity antibody or peptide fragments has culminated in developments of the construction of libraries, phage selection methodologies and fragment affinity maturation. These advances have markedly improved the capabilities of this technique in different fields of science.[10,11]

It is thought that the ability of phage-display technology in identification and characterization of disease specific antigen mimics, is one of the fascinating use of RPL, especially when the pathologic antigen is not known e.g. in autoimmune diseases.

Corresponding Author: Zohreh Jadali, PhD;
Department of Immunology, School of Public Health, Tehran University of Medical Sciences, P.O. Box: 6446, Tehran 14155, Iran. Tel: (+98 21) 646 2268 646 5404, Fax: (+98 21) 646 2267, E-mail: zjadali@razi.tums.ac.ir, or zjadali@yahoo.co.uk

Based on the growing body of evidence, B cells are important contributers in the pathogenesis of a number of autoimmune disorders through different pathways including the synthesis of autoantibodies. Autoantibody production is frequently recognized as a characteristic feature of a variety of autoimmune diseases. It is usually agreed that the persistence of autoantibodies is the consequence of a break in B-cell tolerance which permits autoreactive B cells to escape the selection process.[12,13] These antibodies are invaluable tools for tracking the autoantigen responsible for the initiation and/or perpetuation of autoimme responses.[14-16] There are many reports about the selection of disease specific mimotopes by both monoclonal and polyclonal antibodies related to autoimmune diseases using phage-display technology. For this reason, in this review, we will look at published work to date which employed this strategy for the study of antibody specificity in autoimmune diseases.

Celiac Disease

Celiac disease (CD) is a genetic inflammatory disorder with autoimmune components induced by ingestion of dietary gluten. Gluten is a family of proteins present in wheat. Some of the proteins that make up gluten (the alcohol soluble fraction) are called gliadin. It is the gliadin in gluten that causes the immunological reaction in celiac disease.

Osman *et al*. applied RPL to study antigenic epitopes of antibodies raised against gliadins by immunization of rabbits with gliadins/glutenins (gli/glu). The consensus sequences of the selected peptides contained tri- to pentapeptide stretches matched well homologous regions in gli/glu sequences and mainly localized at the N-terminus of the gliadin suspected to be pathogenic in patients with celiac disease.[17]

In another study, B cell epitopes in gliadin were investigated using the PhD 12-mer library kit and human gliadin antibodies. In this experiment four and one prominent epitopes were identified in α-type and γ-type gliadin respectively. Results from this survey demonstrated that reactivity of human gliadin antibodies was restricted to distinct regions, which is in agreement with previous results on the location of antibody recognition sites in N-terminal repetitive regions. Interestingly, three of the five B cell epitopes are found as identical or homologus sequences in peptides reported as ligands of HLA-DQ2 molecules which have shown strong positive association with the disease.[18]

Screening the FliTrx random dodecamer peptide library with pooled sera from patients with active disease resulted in the identification of a peptide sharing homology with the rotavirus major neutralizing protein VP-7 and the self-antigens tissue transglutaminase (tTG), human heat shock protein 60, desmoglein 1, and Toll-like receptor 4. Among these autoantigens tTG was discovered as a major autoantigen and the authors suggested that in active disease, a subset of anti-transglutaminase antibodies of IgA class recognize the VP-7. They also proposed that rotavirus infection might be involved in the pathogenesis of this disease through a mechanism of molecular mimicry.[19]

Altogether, these surveys have successfully demonstrated the use of phage display technique as a powerful approach to study CD but, further investigations will be required to determine the significance of the identified epitopes in understanding the molecular mechanisms underlying celiac disease.

Myastenia Gravis

Myastenia gravis (MG) is an autoimmune disorder caused by autoantibodies against the nicotinic acetylcholine receptor (AcChoR) on the postsynaptic membrane at the neuromuscular junction and characterized by the weakness and fatigability of the voluntary muscles.

In the first study aimed at delineation of epitope(s) which targeted by autoantibodies in MG using a phage-epitope library an anti- AcChoR monoclonal antibody (mAb) 5.5 which interacts with a conformational dependent epitopes was used. After panning procedure a hexapeptide sequence was identified which interacts specifically with mAb and the synthetic form of this peptide was found to inhibit binding of mAb to the related peptide-presenting phage and to nicotinic acetylcholine receptor and blocking MG-like symptoms that are induced in chicken by passive transfer of mAb.[20]

In another study a mAb 5.14 which reacts with AcChoR in its native and denatured form was employed for screening a hexapeptide phage-epitope library and two different peptide-presenting phages which interact with mAb were selected. AcChoR and synthetic peptides corresponding to the hexapeptide presented by the selected phages inhibited this

interaction. The extended form of the selected peptide increased the affinity of mimotopes to the antibody and efficiently blocked the binding of mAb to both peptide-presenting phages, and to AcChoR.[21]

By screening a 15-mer library with mAb 198 three different peptides were obtained which specifically bound to this antibody. The best of the three peptides which inhibited the binding of mAb to AcChoR was chosen and a cyclic form of this peptide was constructed. The peptide was able to inhibit the binding of mAb to AcChoR with a higher potency than the parent library peptide, blocking the ability of mAb 198 to passively transfer experimental autoimmune MG in rats.[22]

The inhibitory effects of selected peptides suggest that the synthetic form of these peptides may be useful as therapeutic agents for the prevention and modulation of the disease.

Cogan's Syndrome

Cogan's syndrome (CS) is a systemic vasculitis characterized by sensory neural hearing loss and nonsyphilitic interstitial keratitis. Although the etiology of disease is unknown, many researchers believe that CS is an autoimmune mediated disease. To define disease-specific peptide epitopes Lunardi *et al*. used pooled affinity purified IgG from patients' sera for screening a RPL. All the patients' sera were recognized the same selected peptide by enzyme linked immunosorbent assay (ELISA).

This peptide shares homology with different autoantigens such as ribonuclear protein within cell nuclei. This peptide designated SSA/Ro represents an autoantigen in several autoimmune diseases. The selected peptide also shows extensive sequence homology with reovirus III major core protein lambda 1, cell-density enhanced protein tyrosine phosphatase-1 (DEP-1/CD148), which is expressed on sensory epithelia of the inner ear, on the endothelial cells, and connexin 26. The latter is a gap junction protein which is highly expressed in the inner ear. IgG antibodies against the peptide, purified from the sera of patients were able to inhibit proliferation of cells expressing DEP-1/CD148 bound to connexin 26. These antibodies were able to induce the characteristics of the disease in mice.[23]

Autoimmune Thrombocytopenic Purpura

Autoimmune thrombocytopenic purpura (AITP) is characterized by platelet destruction mediated by anti-platelet autoantibodies. Platelet membrane glycoproteins, especially GPIIb-IIIa and GPIb-IX, contain major autoantigenic epitopes, provoking these autoantibodies.

Bowditch *et al*. evaluated the amino acid sequences recognized by plasma autoantibodies purified on platelets from 2 patients with AITP using RPL. They selected two distinct phagotopes (REKAKW and PVVWKN) that bound strongly to plasma autoantibody from patient 1 in a dose-dependent manner. Binding of phage encoding REKAKW to the autoantibodies could be inhibited by a synthetic peptide derived from the GPIIIa cytoplasmic tail but not the other phagotope. Furthermore, this mimotope showed homology with cytoplasmic tail of GPIIIa. When similar studies were carried out with plasma autoantibody from patient 2, a hexapeptide (RELLKM) was identified which was capable of attaching to patients' autoantibody. The selected peptide did not show homology with GPIIIa, however binding of autoantibodies to synthetic GPIIIa peptides was significantly inhibited; therefore this sequences represent a mimotope of the native epitopes.[24]

In another study, Gevorkian *et al*. revealed a panel of phage clones reactive with autoantibodies from other AITP patients by ELISA. None of the peptides defined in this study has been reported previously as a potential epitope for platelet autoantibodies. However, part of these peptides share significant homology with platelet glycoproteins, such as GPIb and GPIIIa. It seems that sera from AITP patients may contain a combination of autoantibodies with a broad range of specificities for a number of autoantigens.[25]

In further evaluation epitope recognition by autoantibodies in AITP, two murine mAbs which were produced against GPIIIa and GPIIb-IIIa and rabbit anti-human platelet polyclonal antibodies were used to select AITP related epitopes using a phage display peptide library. When the specificity of the selected clones were tested by ELISA, seven clones reacted strongly with rabbit anti-human platelet antibodies, and four clones exhibited reactivity when assayed with sera from AITP patients. In addition some homology between platelet GPIIIa and GPIb and selected sequences of phagotopes were identified[26].In conclusion, these results indicate that phage-display peptide library identifies epitopes that bind to anti-platelet antibodies. However further work needs to be done to delineate precisely the role of these mimotopes in the pathogenesis of the disease.

Crohn's Disease

Despite decades of research, the etiology of Crohn's disease (CD) remains unknown. Its pathogenesis may involve a complex interplay between host genetic predisposition, immune dysfunction, and microbial or environmental factors.

One of the major problems for a better understanding of the etiopatogenesis of CD is incomplete information about the nature of antigen(s) which are targeted by the immune system. For the first time the presence of anti-galectin-3 autoantibodies in CD was described by Jensen-Jarolim *et al*. To determine the anti-galectin-3 binding peptide motif, two murine anti- galectin-3 mAbs were used for biopannings of random peptide phage libraries and ligands of two mAbs were randomly selected. Two synthetic peptides of selected phagotopes which present in each group were constructed and their potential for inhibition the binding of anti-galectin-3 autoantibodies to recombinant and native intestinal epitelal galectin-3 were shown.[27]

In another study five different peptides associated with CD were isolated using RPL strategy. Each peptide detected only 15-25% of autoantibodies from CD patients. When the reactivity of 92 patients sera to cocktail of multiple antigenic peptides (MAPs) were analysed by ELISA, 52 of 92 patients (56.5%) were positive. It seems ELISA with a panel of four selected peptides may be a novel serological test for the differential diagnosis of CD.[28]

Insulin Dependent Diabetes Mellitus

Insulin dependent diabetes mellitus (IDDM) has been known as a chronic autoimmune disorder that involves multi-steps leading to the immune destruction of pancreatic beta cells. To determine epitopes, which specifically reacted with sera from patients with IDDM, Mennuni *et al*. used ammonium sulfate precipitated immunoglobulins of sera from two patients for biopannings a mixture of pVIII displayed X_9 and CX_9C libraries. They identified two disease-specific phage clones which reacted with a higher frequency with sera from IDDM patients (26%) and high-risk patients (20%) than the negative sera (4.5%).[29] In another study, the same authors selected one phage clone (phagotope 92) and analysed its reactivity with ELISA. This phagotope was reactive with 30% of newly diagnosed IDDM patients and 41% prediabetic patients. Purified human Igs from patients' sera on this phagotope stained human pancreatic islets suggesting that phagotope 92 mimicked an islet-related autoantigen. Similar reactivity was observed on rat pancreatic islets by testing the rabbit sera immunized with the same phagotope. The newly diagnosed mimotope may mimic a hitherto undefined autoantigen in IDDM because other known diabetes-related autoantigens did not react with anti-phage serum.[30]

Gregori *et al*. aimed to characterize the motif for peptide binding to known IDDM susceptibility locus (I-A^{g7}) in non-obese diabetic (NOD) mouse which is an animal model of human IDDM. Sequencing of phages eluted from I-A^{g7} revealed an enrichment of hydrophobic amino acids (V and P) and positively charged residues (K) at P6 and of positively charged (R and K), aromatic (Y) or hydrophobic (L) residues at P9. The principal anchors defining the phage-derived motif existed in the majority of high-affinity I-A^{g7}-binding peptides from candidate antigens in IDDM.[31]

A novel method to map conformational antibody epitopes reactive with IDDM antibodies has been illustrated by Myers *et al*. Two islet cell mAbs (MICA3 and MICA4) that reacted with the major autoantigen in type 1 diabetes, glutamic acid decarboxylase (GAD65) were employed for screening a mixture of a linear and cyclic random nonapeptide phagmid libraries. This affinity selection resulted in identification of peptide sequences that mimiced the conformational epitopes of GAD65. Alignment of these sequences accompanied by molecular modeling and mutagenesis indicate the involvement of the PEVKEK loop in the conformational MICA3 epitope and support prior studies suggesting that amino acids 511-531 of GAD65 contribute to this epitope.[32] Later on this achievement was confirmed by Fierabracci *et al*. The authors used sera from IDDM patients to screen a RPL and identified five disease specific mimotopes. Among these phagotopes CH1p reacted more strongly with IDDM sera. The CH1p mimotope was detected in somatostatin cells of human islets. In addition experimentally-elicited anti-osteopontin antibodies or human sera which revealed positive reaction for the phagotope identified a similar subpopulation of islet cells. The screening of the lambda gt11 cDNA library identified a specific clone, correspond to human osteopontine.[33] After a few years, in the second set of experiments, Ola *et al*. characterized another member of the disease specific phagotopes (195Dyn) within five selected clones. The peptide, corresponding to human

importin beta, was detected in human islets and recognized as a novel candidate for autoantigen in IDDM.[34]

To gain insight into the molecular determinants associated with insulin antibody (IA) or insulin autoantibody (IAA) idiotypes that could improve the recognition of immunological remission, phage display strategy was employed. This technology was not suitable for determining insulin antibodies according to remission status.[35]

The same authors, used sera from a child with newly diagnosed type 1 diabetes (designated FPP) and from adult-onset type 1 diabetes (T1D) patient with autoimmune polyendocrine syndrome type 2 (APS-II) to screen a RPL in an attempt for selection of mimotopes specific to IAA. The results indicated that phagotope FPP-10 displaced insulin binding in 90% of IAA^+ patients but not in the IA^+ or the APS subject. In contrast, selected phagotopes from the APS-II patient (APS-4) had no specificity for IAA^+ subjects. The authors suggested that anti-idiotype reagents are capable to distinguish childhood-onset T1D-associated IAA^+ from adult-onset T1D that are different from their specificity for human insulin and from anti-idiotope amino acid sequences.[36]

A combination of peptide phage display and molecular modeling have been used to identify the specific ligand(s) for the 96/3 mAb IA-2 autoantibody. This mAb, recognizes a conformational epitope within a protein tyrosin phosphatase (PTP) like protein, which is one of the major autoantigen in human type 1 diabetes and is localized to the secratory granule membranes of islets cells. After screening against mAb the consensus sequence Asn-X-Glu-X-X-(aromatic)-X-X-Gly was identified. Site directed mutagenesis results indicates that Asn^{858}, Glu^{836}, and Trp^{799} are involved in antibody binding reaction.[37]

In summary, a number of these studies have demonstrated that some of the selected peptides can functionally and structurally mimic epitopes of the known diabetes-related autoantigens. However, the ability to mimic a natural epitope does not necessarily correspond to sequence similarity.

Sjogren's Syndrome

Sjogren's syndrome (SS) is a systemic inflammatory autoimmune disease with worldwide distribution, responsible for considerable impact on the patient's quality of life. In order to characterize pathogenetically relevant autoantigen(s), a random dodecamer peptide library was screened with pooled IgG affinity purified from the sera of 12 patients with primary SS. Among the peptides obtained from the last biopanning, one was recognized by the vast majority of patients' sera (72.5%), but none of the sera from control subjects and patients with other autoimmune diseases. This peptide shares sequence homology with an Epstein-Barr Virus (EBV) derived protein and with tear lipocalin, a protein highly expressed in tears and saliva, and with alpha-fodrin, a cytoskeleton protein which is considered as an important autoantigen target in SS. Antibodies against the peptide affinity-purified from the sera of patients recognized the viral protein, tear lipocalin and alpha-fodrin. The authors concluded that EBV infection might contribute to the pathogenesis of SS. In addition they introduced tear lipocalin as a novel autoantigen in SS[38]. This study convincingly validates phage library technology for defining antibody specificity.

Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) is an autoimmune liver disease with profound changes in innate and adaptive immunity, characterised by serum auto-antibodies to mitochondrial antigens. The major mitochondrial autoantigens recognized in the sera of PBC patients are members of 2-oxo-acid dehydrogenase complex (2-OADC) family, including E2 subunit of pyruvate dehydrogenase complex (PDC-E2), E2 subunit of branched chain oxo-acid dehydrogenase complex (BCOADC-E2), and E2 subunit of 2-oxoglutarate dehydrogenase complex (OGDC-E2).

Phage display was used to search for reactivity of a murine mAb C355.1 that reacts with lipoyl domain of PDC-E2. Among 36 selected phagotopes, three putative motifs found were SYP, TYVS and VRH. Amino acid sequence of SYP matched with the same predicted epitope region (SYPPHM) of the inner lipoyl domain of PDC-E2.[39] The immunohistological staining of human combinatorial antibodies of PBC biliary epithelial cells were inhibited by the peptides identified using C355.1.[40]

To identify epitope(s) recognized by antibodies in PBC, random phage-display library was screened using IgG from two patients with PBC and antisera of other origin. Multiple alignment algorithm PILEUP was

exploited to distribute peptides isolated by the screening of library into a guide tree according to their degree of sequence relatedness. PBC-derived sequences primarily clustered to PBC-related branches of guide tree and captured ELISA with affinity-purified anti-PDC-E2 validated alignments of the selected phagotopes using PBC sera.[41]

For the first time, RPL has made it possible to predict the major conformational epitopes of PDC-E2 in PBC based on comparison of the reactive sequences derived by phage library screening with the known NMR structure of an immunodominant epitope in the inner lipoyl domain. In this study, a heptapeptide phage-displayed random peptide library was probed by means of IgG from PBC patients and PBC-specific phagotopes were recognized. These peptides contained two specific motifs, MH and FV (E), which are reactive with affinity purified anti-PDC-E2 by ELISA. The authors predicted a nonsequential residues $_{131}HM_{132}$ and $_{178}FEV_{180}$ that contribute a conformational epitopes. The colocalization of the epitopes identified by C355.1 and the epitope recognized via polyclonal anti-PDC-E2 imply that this epitope is involved to the pathogenesis of PBC.[42]

Additional evidence that the motifs MH and FV (E) contribute to the development of this disease are the result of biopanning of phage-displayed library with IgG from three patients with PBC and from three patients with autoimmune cholangitis (AIC). Surprisingly, the isolated phagotopes from these patients contained the motifs MH and FV, however sera from AIC did not have antibodies to PDC-E2, but instead antibodies, to one or another set of nuclear antigens were seen in both diseases. Since, the selected phagotopes reacted with anti-PDC-E2 it is possible that similar autoantigen targeted in PBC and AIC.[43]

To elucidate the structural features of B cell epitope within the PDC-E2 subunit (amino acids 91-227) which is believed to include the residue K_{173} that binds the essential lipoic acid cofactor for the enzyme, phage libraries were screened using IgG from a PBC serum. The results indicated that reactive peptide with affinity-purified anti-PDC-E2 contained MH or FV/E motifs. The authors proposed that a conformational epitope formed from at least two separate regions $H_{132}M_{133}$ and F_{178},V_{180} confirmed by site-directed mutagenesis. One of the most surprising finding of this investigation was that the major epitope of PDC-E2 appeared not to include K_{173} that has long been thought to be critical component of the B cell epitope.[44]

In conclusion, these studies utilised phage-display technology to identify an antigenic determinant recognized by both monoclonal and polyclonal antibodies. Some of the selected peptides are immunologically reactive and allow the prediction of the structure of disease-related epitopes.

Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is another type of autoimmune diseases of the liver which is not only different regarding its clinical profile but also differs in diagnostic strategy, therapeutic regimen and probability of remission, as well as its association with other immune-mediated diseases and cancer. To characterize the reactivity of anti-liver cytosol type 1 (anti-LC1) autoantibodies which are specific for AIH, a RPL was screened using HCV+/LC1+ patients' sera. As a result five different clones were identified. These peptide sequences were compared with human formiminotransferase cyclodeaminase (hFTCD), a 62 KD intracellular enzyme found in cytosol of hepatocytes. Alignment of the five peptides displayed by selected phagotopes with the amino acid sequences of hFTCD, showed similarities between hFTCD and all mimotopes.[45]

Mixed Cryoglobulinemia (CryoII)

Mixed cryoglobulinemia (MC) type II is an autoimmune disorder associated with hepatitis C virus and characterized by circulating cold-precipitable immune complexes composed of polyclonal immunoglobulin IgG and monoclonal IgM rheumatoid factor (RF). With the aim of identifying phagotopes reacting with antibodies common to CryoII/HCV, IgM purified from cryoprecipitate of patients and used for screening of library. A consensus sequence HPL and/or an extended consensus sequence HPLAP were shared by 70% of the identified phages. This motif was recognized by a large proportion of the sera positive for both HCV and CryoII. After homology search of amino acid sequence there was no sequence homologous with HCV, but homology to the immunoglobulin-like domain of the human lymphocyte activation gene 3 product (LAG3) was observed. Antibodies obtained after immunization with LAG3 recognized the synthetic LAG3 peptide, the native form of LAG3 and the phages. In addition, reaction between IgM of

CryoII$^+$/HCV$^+$ and MAP-LAG3 was observed. These findings indicate that LAG3 could represent a novel autoantigen in MC.[46]

The data from this survey confer experimental support for phage-display approach to identify a new autoimmune-related epitope.

Systemic Sclerosis

Systemic sclerosis (SSc), also known as scleroderma, is a complex connective tissue disease, characterized by an abnormal immune activation, a vasculopathy and a fibrosis of the skin and of multiple internal organs.The cause and pathogenesis of SSc are only partially defined and it seems identification of molecules that are recognized by immune system plays a major role in the understanding of the pathogenesis of disease and permits to introduce novel treatment that improves the management of some aspects of the disease. For this reason, to define pathogenetically relevant autoantigen targets in SSc a dodecamer random peptide library was screened with pooled IgG derived from a panel of 90 patients with SSc. Using this technique a peptide was identified that was specifically recognized by a large percentage (93%) of the individual patients' serum IgG by both direct and competitive ELISA and showing strong sequence homology to heterogeneous nuclear ribonucleoprotein, fibrillarin and human cytomegalovirus (HCMV) late protein UL94. Affinity-purified anti-peptide IgG from patients' sera recognized the viral product and autoantigens. In addition these antibodies were able to induce endothelial cell apoptosis through their interaction with the cell surface integrin-NAG-2. The authors of this arthicle suggest a causal link between human cytomegalovirus virus infection with autoimmunity in SSc.[47]

The results of this are examples of the potency of phage-display technology for introducing a previously unknown mechanism for the ethiopathogenesis of disease.

Vitiligo

Vitiligo is an acquired pigmentary skin disorder that results from the selective destruction of melanocytes. Its etiology is unknown, but might involve genetic factors, autoimmunity, neurologic factors, toxic metabolites, and lack of melanocyte growth factors. Despite the efforts made by a number of research workers to identify the autoantigen(s) that induce the corresponding antibodies, the target autoantigen in vitiligo is not known. To identify the peptides that, as epitopes, react specifically with the autoantibodies produced by patients with vitiligo, affinity selection experiments and screening by a PIII-displayed RPL was used and revealed two phagotopes that showed specific reaction with the antibodies present in the pooled sera from the patients with vitiligo. The reaction of the phagotopes with total immunoglobulin by patient sera was significantly higher than controls. In addition, analysis of the antibody isotypes revealed that the dominant immunoglobulin isotype was IgG, and there was a significant statistical difference between the patients and normal control in frequency of this isotype.[48,49]

It seems that further research is needed to determine the potential role of selected peptides in vitiligo pathogenesis.

Antiphospholipid Syndrome

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of a heterogeneous group of antiphospholipid antibodies. These antibodies are collectively termed as antiphospholipid antibodies (aPL). Many recent studies, indicate that aPL are in fact anti-β2-GPI antibodies directed against an epitope which is expressed when β2-GPI is bound to anionic phospholipid or another suitable surface.

Screening of phage-displayed random peptide libraries with anti-β2-GPI antibodies from a patient with APS led to the identification of a phage that specifically bound to these antibodies. Phage derived peptide was optimized and a 14-mer peptide (LJP 685) that maintained the binding of the original phage constructed. A tetravalent form of LJP 685 used as a tolergen and induced a dose dependent decrease in antibody levels in mice previously immunized and boosted with LJP 685 coupled to the carrier keyhole limpet hemocyanin. These data suggest that identification and construction of such mimotops are potentially suitable for production of a tolergen drug.[50]

Koike *et al*. made another attempt to identify the cryptic epitope(s) of monoclonal anti-β2-GPI antibodies using RPL. After the last round of panning ten clones randomly selected for evaluation of binding to Cof-18, -20 and -21 antibodies which recognized the native structure of human β2-GPI and their epitopes located on domain V, III and IV, respectively. Phage

clones selected for binding to these antibodies contained consensus motifs found on the structural model of human β2-GPI. To further this study epitope mapping of three monoclonal anticardiolipin antibodies (EY1C8, EY2C9 and TM1G2) was carried out by phage display strategy and sequence similarity between the consensus sequences of selected phagotopes and domain IV of β2-GPI was identified.[51]

In another study three hexapeptides were selected during the screening of phage display library with three anti- β2-GPI mAbs called ILA-1, ILA-3, and H3. All of these peptides were found to react with mAbs and had the potential to induce experimental APS and endothelial cell activation. The elongated form of these peptides was able to inhibit the biological functions of the corresponding anti- β2-GPI mAbs. For example endothelial cell activation induced by anti-β2GPI and binding of these mAbs to β2-GPI were inhibited by these peptides.[52]

Visvanathan and colleagues screened 17 phage-disply peptide libraries against CL15 monoclonal anticardiolipin antibody (aCL) and revealed a peptide (CL154C) which would react with CL15 and had a potency for inhibition of cardiolipin binding to this mAb. More than half of APS patients were positive for the presence of CL154C reactive IgG.[53] The existence of cryptic epitopes on a hidden site of domain IV of β2-GPI have been reported in APS. Phage display strategy was used for characterizing antigenic determinant specific for anti- β2-GPI antibodies. The epitopic regions identified by this study primarily made up of hydrophobic amino acids stretches located on two discontinuous sequences in domain IV. Domain V covered these groups of amino acids revealing their hidden nature.[54]

Peptide ligand of a mAb with procoagulant activity (HL-5B) was identified by screening random phage-displayed libraries. This peptide was able to bind mAb in a concentration dependent manner and prevent the binding of HL-5B to cardiolipin and phosphatidylserin. Furthermore, when reactivity of IgG and IgM antibodies from patients with APS were analysed by ELISA, more than half of patients (67%) were able to react with peptide.[55]

These findings not only validate the technology of RPL for epitope mapping but also help in improving therapeutic strategies for the treatment of APS.

Graves' Disease

Graves' disease (GD) is a well-known organ-specific autoimmune disease, in which stimulatory anti-thyrotropin (TSH) receptor antibodies cause hyperthyroidism and diffuse goiter. Byun and colleagues utilized phage display technology to identify the epitopes of two human monoclonal thyroid-stimulating antibodies (B6B7 and 101-2). Alignment of deduced amino acid sequences of peptides resulted in identification of SPWTLGA and TQWNMQH sequences that bound to B6B7 and 101-2 mAbs respectively and displayed specificity for their relevant antibodies. The SP and TQ motifs found in the N-terminal of sequences mentioned above were observed in extracellular domain of human TSHR. These peptides were bound with specificity to the mAbs and IgG of the patient and inhibited cAMP synthesis induced by the mAbs as well as Graves' IgG in CHO cells expressing human TSHR.[56] Following above-mentioned study, Chan *et al*. affinity purified IgG that binds to extracellular domain of human TSHR as an important target for TSAbs. These antibodies which induced cAMP synthesis and had inhibitory activity on TSH binding to TSHR were used for screening of library. Only one of the selected peptides was further analysed because of limited accessibility to the GD IgG. A low concentration of this peptide inhibited the action of TSAb, however it did not inhibit TSH induced cAMP synthesis.[57] In another effort, phage-displayed peptide technology pursued by sequence alignment on the TPO molecule employed to localize discontinuous epitopes on human thyroid peroxidase (hTPO). The outcome of these experiments were identification of three regions (353-363, 377-386, and 713-720) belonging to the myeloperoxidase-like domain and one (766-775) was found on complement control protein-like domain of TPO as being involved in the immunodominant region. Interestingly, these regions were implicated in the binding to TPO of Hashimoto's and graves' sera, positive for anti-TPO autoantibodies.[58]

In summary, it seems mimicry epitopes can be identified via screening of RPL and some of them share sequence similarity with the corresponding natural epitopes. In the future these peptides may be quite useful for diagnosis, prognosis and development of immunosuppressive agents for GD.

Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a systemic autoimmune disease characterized by chronic synovitis

and bone damages, which consists of joint destruction and systemic osteoporosis. The importance of B cells in the pathogenesis of RA as highlighted by the presence of a number of autoantibody systems have now been illuminated in RA and their clinical associations with the pathogenesis of disease has also been postulated. Evidence for the presence of antibodies to native and denatured type II collagen (CII) in patients' sera with RA and synovial fluids together with collagen-anticollagen complexes in synovial fluids suggested the possible significance of immune response to type II collagen. Phage-display epitope mapping of monoclonal antibody C1 against type II collagen (CII-CI) led to identification of peptides containing the sequence RLPFG occurring in EBV nuclear antigen, EBNA-1. This finding is consistent with previous literature supports a role of EBV in the induction of RA because of increased antibody titers to EBNA-1 in patients' sera.[59] In another study, Davies *et al*. examined whether or not variation exists in the peptide sequences and its immunoreactivity among 12 selected phagotopes using mAb CII-C1. Direct and inhibition ELISA methods showed all of 12 phagotopes were reactive with CII-C1, however this reactivity differed according to assay format and peptide sequence. Furthermore the phage-expressed peptide sequences were grouped into clusters of similar peptides using the computer-based sequence alignment algorithm PILEUP. The results indicated that variations in reactivity among the phagotopes were often in accord with the alignment of the peptide sequences.[60] In the same year, these authors after screening of phage-displayed RPL on anti-type II collagen antibodies published another article and showed 5 phagotopes out of 17 had homology to EBNA-1. Furthermore, the reactivity of patient sera of whom 26 had antibodies to native type II collagen with the peptide (RRLPFGSQM) were tested by ELISA. The selected phagotope reacted with 16% RA sera but not with the control sera. These data attest that this phagotope functions as a mimic of an epitope in type II collagen.[61]

To verify and extend the results of previous study a second mAb, CB268, that identifies the similar conformational epitope as CII-C1, was employed for screening the same libraries. Most of the phage-derived CB268-binding peptides have either two or three basic residues, and often a hydrophobic residue positioned in the first four amino acids. These findings correspond to the results of prior study. This investigation indicated that two mAbs can target a single epitope on one antigen (CII).[62]

Sera from RA patients were used as a source of polyclonal antibodies for screening of libraries. This procedure resulted in selection of five phage clones and comparison of their binding using ELISA indicated that all of selected phages react more frequently with patients' sera than control subjects. A data base search with one of the selected peptide named pep1 revealed a significant homology with EBNA-1. These findings therefore suggest a possible role of EBV in the pathogenesis of RA.[63] To investigate the antibody specificities in the synovial fluids (SF) of RA patients the same authors used a nonapeptide phage library. The sequence data showed the motifs recognized by SF antibodies were different from antibodies isolated mimotopes from the patients' sera. It is likely that the B cell expansion in the joint fluid synovium originated by stimulation of a local specific antigen. Although, a common SF specificities was observed among patients with RA.[64] In previous study GDIA motif occurred in one of the selected phagotopes that shares homology with collagen type IX and XII, EBNA-4 nucleoprotein and parainfluenza virus nucleocapsid protein. Antibody response against synthetic forms of immunoselected sequence which contained the consensus sequence with GDIA, glycin-rich cell wall protein and proteus protein derived peptide were analysed using ELISA. These results indicated that sera and SF from RA patients had higher anti-peptide reactivity than did SF and sera from control individuals.[65]

The elevated levels of autoantibodies against TNFα were reported in RA. To characterize epitopes recognized by these antibodies, TNFα affinity-purified polyclonal autoantibodies were used for biopanning experiments. Among the 63 randomly selected phagotopes, there were only nine different sequences which demonstrated the immune response of patients to TNFα is restricted. These peptides contained a conserved 3-amino acid motif (Ser-Ser-X motif, where X is either Pro/Leu or Phe. Furthermore, the peptide sequences of two selected phagotopes correspond to residues 11-13 and 36-38 of TNFα which the former residues involves in binding to TNFα receptor.[66] The association between MHCII and some autoimmune diseases including RA is well established. To characterize the DQ4-binding peptide sequence which is a frequent haplotype in Japanese patients with RA, a 15-mer random peptide library was employed and high-

affinity ligands binding to HLA-DQ4 were identified. This study indicated that two important anchor residues (VxxxxxxxR, where x is any amino acid) play a pivotal role in binding to DQ4 and showed that high affinity MHC binding can be achieved if Arg present at position 9. It seems also attractive that multiple fragments of type II collagen as a candidate autoantigen in RA, can bind to RA-susceptible HLA haplotype DR4 and at the same time is rich in Arg.[67] Screening of phage display libraries with a high affinity IgM rheumatoid factor expressing the VkIIIa-dependent 4C9 idiotype (B'20) led to identification of two motifs. Binding of selected phagotopes to B'20 was inhibited by both IgG and 4C9 anti-idiotypes. It is thought that this binding is RF specific because in the absence of RF specificity both in mutants of B'20 and in 4C9 positive cell lines their binding to the phage were prohibited.[68]

FKN-E12 mAb is produced by B cell clone derived from SF of a patient with sero-negative RA and consists of IgG1 lambda. By screening a heptamer random peptide library only one sequence was recognized (RASFp1 = HLTFGPG). Five amino acids (TFGOG) of this sequence occur in all human k chains generated by rearrangement of Vk-gene with Jk3. Three human Ig k light chains also contain the hexamer LTFGPG.[69] To answer this question whether or not sera from autoimmune (RA and SLE) and non-autoimmune inflammatory diseases contain IgG binding to RASFp1 a 15-mer-peptide was synthesized. Antibodies binding to this peptide which contained RASFp1 were analysed by ELISA. The results demonstrated that 45% of patients' sera suffering from RA and SLE had RASFp1-binding antibodies while only 5% of normal controls and patients with non-autoimmune inflammations were positive.[70]

A new panning procedure used by Hansen *et al*. for identification of the specificities of anti-nuclear antibodies (ANA) in patients with juvenile RA (JRA). After immunoscreening, 71 positive phage clones were sequenced and the resulting peptides were divided into 2 groups, while some of which exhibited common core motifs. Antibodies in patients' sera were affinity purified against phage displaying peptide and were able to stain perinuclear and nuclear region of HEp-2 cells. Furthermore the reactivity of ANA positive sera to some of the core motifs were more frequent as compared to ANA-negative JRA patients. Identified motifs were then used to search the SwissProt database and a significant homology was found with a number of human nuclear proteins and proteins from potential infectious agents which may act in tolerance breakdown and initiating autoimmunity.[71] In vivo phage display selection was also employed for identification of synovium-specific homing peptides. This effort led to isolation of synovial homing phages expressing particular peptides that showed specific adhesion to synovial but not skin or mouse microvascular endothelium (MVE). Noteworthy, one peptide (CKSTHDRLC) retained synovial homing specificity in the form of a phage-bound peptide or as a free synthetic peptide. The latter was blocked in vivo binding of the parent phage to the cognate synovial MVE ligand.[72]

Taken together, these studies reconfirmed the usefulness of RPL in identifying peptide antigens that might be part of causative agents in autoimmune diseases.

Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system with a presumed autoimmune etiology. RPL was employed in order to acquire more information about the causative (auto)antigen in MS.

The first attempt was screening of library against cerebrospinal fluid (CSF) which led to selection of mimotopes. These peptides were recognized with equal frequency by sera from both patients and control subjects and suggested the likelihood that peptides expressed by the phagotopes mimic the epitopes of ubiquitous antigens that are also antigenic in normal subjects.[73]

In the next year, Dybwad *et al*. published another article after screening the library with human CSF. They selected peptides expressing two peptide motifs S (S/T/Q) (R/S) (N/G) FP and PRn (G/P) FF. Sequence alignments and homology searches revealed a significant homology with collagen proteins, and proteins from viruses such as herpes simplex virus, human cytomegalovirus and human papillomavirus.[74]

For further characterization of the phagotopes recognized by IgG fraction in cerebrospinal fluid (CSF) from MS patients phage display strategy was employed. The selected phagotopes were grouped in six families each composed of different isolates. The selected phagotopes have been used as reagents to assess the specificity of CSF IgG in MS patients. The data showed different CSF antibody specificities in

diferrent patients and the presence of anti-phagotope antibodies in both sera and CSF of patients. Furthermore, the frequency of anti-phagotope antibodies in the sera of MS patients was similar to the control population. This confirmed the results of previous study and suggested that natural antigen(s) identified by CSF antibodies is frequent in the general population.[75]

RPL approach was again used for identification of disease specific phagotopes reacting with CSF-enrich antibodies. This technique resulted in the recognition of phagotopes reacting with CSF-enrich antibodies. A panel of 55 CSF from MS patients was tested for reactivity with the selected ligands by ELISA. The results demonstrated none of CSF reacted with any of the phage and supporting this assumption that the set of CSF-enriched antibodies is completely patient specific.[76]

It seems the specificity of the antibodies in the oligoclonal bands (OB) within the CNS of patients is generally directed against the causative agent(s) of disease, thus identification of epitops as target of these antibodies allow a deeper understanding of the immune mechanisms of disease. For this reason, CSF IgG antibodies from MS patients were subjected to panning experiments and resulted in recognition of phagotopes which several of them expressed a linear motif with RRPFF sequence. This motif showed homology with EBNA-1 and αB crystain and surprisingly increased level of αB crystain has been reported in various neurological disorders.[77]

In subsequent study, these authors employed Western blotting and isoelectric focusing to overcome the non-specifity problems associated with peptides selected from RPL. These methods provided the means to detect the co-migration of antigen specificic OB with individual total IgG bands.[78] After a few years, Cortese *et al*. chose MS17 phage for further analysis. This phagotope was selected for two major reasons. The first was for its good reactivity towards CSF antibodies and the second was for its ability to react mainly with the patients' sera. They immunized rabbit with MS17 mimotope which contained a common KPPNP motif and showed a significant homology with an exposed region of the glycoprotein B of HSV-1. Using both ELISA and affinity-purified antibodies they confirmed the anti-mimotope IgG cross-reactivity with a brain specific protein and the surface glycoprotein gB of HSV-1.[79]

In another attempt, a pentadecapeptide library was applied for screening of sera and CSF taken from the same MS patients. The three mimotopes isolated with patients' sera and their corresponding specific synthetic peptides were tested for their ability to bind to individual sera from patients in ELISA studies. The results indicated that they were not disease-specific. On the other hand, a mixture of 4 mimotopes isolated from CSF, permitted the recognition of specific antibodies in CSF of 21 out of 60 MS patients. Two mimotopes which selected from patients' CSF showed amino acid similarities with envelope regions of multiple-sclerosis-associated retrovirus and the related endogenous retrovirus HERV-W. Interestingly, there is some evidence about the presence of MSRV virions in the CSF at the onset of MS and its association, not only with disability accumulation, but also with a higher rate of clinical re-exacerbations.[80]

Recombinant antibodies (rAbs) generated from B cells in the CSF of multiple sclerosis were used to probe a random phage-displayed peptide library. A group of antibody binding peptides were identified in this study and their specificity to rAbs was confirmed by inhibition assay. Interestingly, these peptides were also identified by the native IgG in the CSF of MS patients from which the rAb was cloned. These data also suggested that MS rAb imitates the specificity of IgG in the CSF of MS patients[81]. Furthermore, previous authors improved identification of complete potential phage peptides for MS rAbs using high-throughput method. The advantages of the method are rapidity, lack of requirement for large scale phage purification and immediate characterization of a great number of phage clones.[82]

These results demonstrate that RPL is a suitable means for the recognition of the natural antigens binding to antibodies present in the biological fluids of patients.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a complex polygenic autoimmune disease associated with a chronic activation of the immune system. B cells were implicated in lupus pathogenesis and autoantibodies and immune complexes have long been known to be centrally involved. The nearly universal presence of autoantibodies is a prominent unifying feature in this autoimmune disease. Despite intense efforts by the researchers to characterize the nature of the antigenic

target(s) of lupus antibodies, the finer aspects of its ligands remained controversial. Sibille *et al*. carried out a survey using three reactive anti-DNA mAbs (F14.6, F4.1 And J20.8) which had been obtained from (NZB×NZW)F1 mice as a primary model of lupus like syndrome. Peptides reacting with the F14.6 and J20.8 mAbs were isolated by screening a constrained random peptide library. Phages selected on F14.6 did not cross-react with J20.8 and vice versa. A subset of J20.8-specific peptides also recognized by serum Abs from SLE patients and immunization of BALB/c mice with some of selected phages resulted in the elicitation of anti-DNA responses. The presence of negative charge and aromatic rings in peptides specific for F14.6 implicates the possible role for molecular mimicry in antibody/DNA interactions.[83] By screening of phage peptide library using a murine IgG2b monoclonal anti-dsDNA antibody (R4A) a peptide surrogate for dsDNA was identified. This peptide, DWEYSVWLSN, and the smaller part of it, DWEYS, were reacted specifically to the pathogenic R4A antibody, which was bound to glomeruli of nonautoimmune mice. Binding of R4A to dsDNA inhibited by either the L or the D configuration of DWEYS and injection of D peptide form to mice inhibited kidney deposition of R4A. Therefore, this peptide may eventually offer a promising therapeutic option for the treatment of SLE.[84] Immunization of nonautoimmune BALB/c mice with DWEYSVWLSN augmented the production of IgG anti-ds DNA antibody, anti-histone antibodies and anti-cardiolipin antibodies, supporting the notion that there are shared epitopes on these antigens. BALB/c mice also develop immunoglobulin deposition in renal glomeruli may be susceptible to the pathogenicity of peptide-induced autoantibodies. These results indicated that a peptide could mimic DNA and elicit pathogenic antibody response in nonautoimmune animals.[85] To gain further insights into the molecular basis of the generation of autoreactivity in peptide-immunized mice a panel of 24 monoclonal IgM antibodies were developed from splenocytes collected from immunized mice. Analysis of these antibodies which were reactive with peptide and/or autoantigen led to the recognition of three groups of Abs. The first group recognized only the peptide. The second group was found to cross-react with one or more autoantigens and the third group did not bind to peptide. Interestingly, a similar gene families and specific VH-VL combinations used by both hybridomas binding DNA (which was generated from peptide-immunized mice) and anti-DNA response in lupus prone mice. Taken together, these findings imply the value of phage display peptide libraries in generating true molecular mimics.[86] To assess whether there are differences in B cell responses to DWEYSVWLSN between autoimmune and nonautoimmune mice, autoimmune-prone mice (NZB×NZW)F1(B/W), were used for immunization. Comparison of anti-peptide responses among peptide-immunized and unmanipulated B/W mice indicated a marked increase in IgG anti-ds DNA, anti-laminin and anti-cardiolipin antibodies and a more sever kidney disease in peptide immunized mice. There is a possible a role for this peptide in the accelerated autoimmune manifestations in genetically susceptible mice.[87] Sharma *et al.* continued the study to determine the R4A peptide specifications. Analysis of the binding of human monoclonal and polyclonal antibodies to DWEYS motif indicated the fact that the peptide was able to inhibit DNA binding by four immunoglobulin (Ig) G and two IgM human monoclonal antibodies. Greater inhibition of IgM could be achieved in comparison to IgG, may reflect alterations in antibody fine specificity following affinity maturation. Application of monomeric and multimeric forms of the peptide in inhibition ELISA indicated an octameric form of the peptide, which was inhibited by serum anti-DNA reactivity in 10 SLE patients.[88] Because of the importance of anti-dsDNA in SLE, there is an increasing trend towards identification of the putative antigens for these antibodies. A successful attempt was made by Sun *et al.* They used monospecific SLE anti-dsDNA to select phage from RPL and introduced a peptide motif, RLTSSLRYNP, which could be recognized by 88% of anti-dsDNA antibody-positive SLE sera. Enzyme inhibition assay demonstrated the inhibitory effects of dsDNA, single stranded(s) DNA and native RNA on the motif binding activity of SLE sera. This motif can act as an immunogen in rabbits and induces the production of anti-peptide antibodies which cross-react with the peptide, ds and ssDNAs.[89] The occurrence of nucleosome-specific antibodies in SLE has been reported frequently. This antibody specificity developed prior to the appearance of anti-DNA and anti-histone antibodies. Therefore determination of ligand-binding specificity of these antibodies is very important. To obtain information about the B cell epitope recognized by anti-nucleosome antibodies Dieker *et al*. used the nucleosome-specific mAb (#32)

as the target to screen the 10-mer phage display peptide. They identified a mimotope not only bound to the mAb but also bound to a series of nucleosome-specific, anti-DNA and anti-histone antibodies.[90] In another effort, screening of a 15 mer peptide library with an affinity purified anti-dsDNA antibody resulted in identification of a 15 mer peptide ASPVTARVLWKASHV. A good correlation was found between the synthetic form of peptide with dsDNA in the binding reactivities with human serum antibodies to dsDNA. This peptide was recognized by all 41 patients sera tested and competed effectively in vivo with dsDNA and ssDNA for binding to anti-DNA antibodies. These observations proposed the peptide mimic, ssDNA and dsDNA share similar antigenic structure.[91]

Altogether, the methods described in these studies showed that phage-display libraries represent a novel approach to identify antigen specificity.

CONCLUSION

B cells influence autoimmunity through multiple pathways. These include the ability of B cells to generate self-reactive antibodies, secretion of inflammatory cytokines, presentation of antigens, enhancement of T cell activation, and production of ectopic lymphogenesis.[92,93]

Although all of these mechanisms are involved in B cell autoimmunity to some extent, autoantibody production has received more attention by the researchers. In some instances (e.g. GD), these autoantibodies bind self-antigens and can interfere with normal cellular activities and harness immunological effector mechanisms to evoke authoimmune responses.[13] Furthermore autoantibodies provoke disease via Fc-mediated activation of complement system.[94] It is also possible that the activation of Fc receptors by autoantibodies and immune complexes is effective for the induction of autoimmune diseases.[13]

One of the major limitations in the study of many autoimmune diseases is lack of information about the structure of pathological antigen. Therefore, the definition of (auto)antigen is principal in a number of situations, including: the understanding of the immunopathogenic mechanisms of disease, development of diagnostic assay and design of prophylactic strategies. In recent years a number of different approaches have been used for designation of high affinity ligands for both T and B cell epitopes. Among them phage-display technology offers major advantages. In this technique thousands to millions of peptides can be screened in one assay. Furthermore, it allows the identification of "disease related" mimotopes when the etiological agent is unknown. This technology has other advantages which include needing a modest amount of effort, time and expense for selection of peptide ligands.

For these reasons, the scientists have been encouraged to apply the phage display technology to study autoimmune diseases. Panning the phage libraries against antibodies has confirmed to be very successful for selection and identification of mimic epitopes. Nontheless, not the entire of the selected phage clones are antigenic. Therefore, the specificity of the clones must be tested with different assays including competitive experiments. These assays recognize those that may need further focus of attention as binders, agonists or antagonists, antigens or immunogens. The results of these various assays demonstrate whether a mere phage insert qualifies as an antigenic mimotope.[95]

In summary, these studies highlight the power of phage-display approach in tracing disease-related epitopes. Identified epitopes or mimotopes could contribute to the elucidation of the molecular basis of the biological recognition and may be useful for diagnostic and prognostic assessments. Furthermore, these could even be exploited for recognizing unidentified etiologic agents. Therefore, it seems that this strategy can address many of the most desirable purposes of clinical immunologist, although other technologies are needed to delineate and stabilize the immunologic structure.

REFERENCES

1. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 1985; 228(4705):1315-7.
2. Devlin JJ, Panganiban LC, Devlin PE. Random peptide libraries: a source of specific protein binding molecules. Science 1990; 249(4967):404-6
3. Scott JK, Smith GP. Searching for peptide ligands with an epitope library. Science. 1990; 249(4967):386-90.
4. Cwirla SE, Peters EA, Barrett RW, Dower WJ. Peptides on phage: a vast library of peptides for identifying ligands. Proc Natl Acad Sci U S A 1990; 87(16):6378-82.

5. Smith GP, Petrenko VA. Phage Display. Chem Rev 1997; 97(2):391-410.
6. Zwick MB, Shen J, Scott JK. Phage-displayed peptide libraries. Curr Opin Biotechnol 1998; 9(4):427-36.
7. Cortese R, Monaci P, Nicosia A, Luzzago A, Felici F, Galfre G, et al. Identification of biologically active peptides using random libraries displayed on phage. Curr Opin Biotechnol 1995; 6(1):73-80.
8. Irving MB, Pan O, Scott JK. Random-peptide libraries and antigen-fragment libraries for epitope mapping and the development of vaccines and diagnostics. Curr Opin Chem Biol 2001; 5(3):314-24.
9. Hoess R, Brinkmann U, Handel T, Pastan I. Identification of a peptide which binds to the carbohydrate-specific monoclonal antibody B3. Gene 1993;128(1):43-9.
10. Sergeeva A, Kolonin MG, Molldrem JJ, Pasqualini R, Arap W. Display technologies: application for the discovery of drug and gene delivery agents. Adv Drug Deliv Rev 2006; 58(15):1622-54.
11. Manatunga V, Sanati H, Tan P, O'Brien PA. Maceration of plant tissue by fungi is inhibited by recombinant antipectinase antibodies. Eur J Plant Pathol 2005; 112:211-20.
12. Ding C, Yan J. Regulation of autoreactive B cells: checkpoints and activation. Arch Immunol Ther Exp (Warsz) 2007; 55(2):83-9.
13. Martin F, Chan AC. B cell immunobiology in disease: evolving concepts from the clinic. Annu Rev Immunol 2006; 24:467-96.
14. Tuscano JM, Harris GS, Tedder TF. B lymphocytes contribute to autoimmune disease pathogenesis: current trends and clinical implications. Autoimmun Rev 2003; 2(2):101-8.
15. Chan OT, Madaio MP, Shlomchik MJ. The central and multiple roles of B cells in lupus pathogenesis. Immunol Rev 1999; 169:107-21.
16. Murakami M, Honjo T. Transgenic mouse models for B-cell dominant autoimmune diseases. Curr Opin Immuno. 1997; 9(6):846-50.
17. Osman A, Uhlig H, Thamm B, Schneider-Mergener J, Mothes T.Use of the phage display technique for detection of epitopes recognized by polyclonal rabbit gliadin antibodies. FEBS Lett 1998; 433(01-2):103-7.
18. Osman AA, Gunnel T, Dietl A, Uhlig HH, Amin M, Fleckenstein B, et al. B cell epitopes of gliadin. Clin Exp Immunol 2000; 121(2):248-54.
19. Zanoni G, Navone R, Lunardi C, Tridente G, Bason C, Sivori S, et al. In celiac disease, a subset of autoantibodies against transglutaminase binds toll-like receptor 4 and induces activation of monocytes. PLoS Med 2006; 3(9):e358.
20. Balass M, Heldman Y, Cabilly S, Givol D, Katchalski-Katzir E, Fuchs S. Identification of a hexapeptide that mimics a conformation-dependent binding site of acetylcholine receptor by use of a phage-epitope library.Proc Natl Acad Sci U S A 1993;90(22):10638-42.
21. Barchan D, Balass M, Souroujon MC, Katchalski-Katzir E, Fuchs S. Identification of epitopes within a highly immunogenic region of acetylcholine receptor by a phage epitope library.J Immunol 1995;155(9):4264-9.
22. Venkatesh N, Im SH, Balass M, Fuchs S, Katchalski-Katzir E. Prevention of passively transferred experimental autoimmune myasthenia gravis by a phage library-derived cyclic peptide. Proc Natl Acad Sci U S A 2000; 97(2):761-6.
23. Lunardi C, Bason C, Leandri M, Navone R, Lestani M, Millo E, et al. Autoantibodies to inner ear and endothelial antigens in Cogan's syndrome. Lancet 2002; 360(9337):915-21.
24. Bowditch RD, Tani P, Fong KC, McMillan R. Characterization of autoantigenic epitopes on platelet glycoprotein IIb/IIIa using random peptide libraries. Blood 1996; 88(12): 4579-84.
25. Gevorkian G, Manoutcharian K, Almagro JC, Govezensky T, Dominguez V. Identification of autoimmune thrombocytopenic purpura-related epitopes using phage-display peptide library. Clin Immunol Immunopathol 1998; 86(3):305-9.
26. Gevorkian G, Manoutcharian K, Govezensky T, Cano JA, Dominguez V, Santamaria H, et al. Identification of mimotopes of platelet autoantigens associated with autoimmune thrombocytopenic purpura. J Autoimmun 2000; 15(1):33-40.
27. Jensen-Jarolim E, Neumann C, Oberhuber G, Gscheidlinger R, Neuchrist C, Reinisch W, et al. Anti-Galectin-3 IgG autoantibodies in patients with Crohn's disease characterized by means of phage display peptide libraries. J Clin Immunol 2001; 21(5):348-56.
28. Saito H, Fukuda Y, Katsuragi K, Tanaka M, Satomi M, Shimoyama T, Saito T, Tachikawa T. Isolation of peptides useful for differential diagnosis of Crohn's disease and ulcerative colitis. Gut 2003; 52(4):535-40.
29. Mennuni C, Santini C, Dotta F, Farilla L, Di Mario U, Fierabracci A, et al. Selection of phage-displayed peptides mimicking type 1 diabetes-specific epitopes. J Autoimmun 1996; 9(3):431-6.
30. Mennuni C, Santini C, Lazzaro D, Dotta F, Farilla L, Fierabracci A, et al. Identification of a novel type 1

diabetes-specific epitope by screening phage libraries with sera from pre-diabetic patients. J Mol Biol 1997; 268(3):599-606.

31. Gregori S, Bono E, Gallazzi F, Hammer J, Harrison LC, Adorini L. The motif for peptide binding to the insulin-dependent diabetes mellitus-associated class II MHC molecule I-Ag7 validated by phage display library. Int Immunol 2000; 12(4):493-503.
32. Myers MA, Davies JM, Tong JC, Whisstock J, Scealy M, Mackay IR, Rowley MJ. Conformational epitopes on the diabetes autoantigen GAD65 identified by peptide phage display and molecular modeling. J Immunol 2000; 165(7):3830-8.
33. Fierabracci A, Biro PA, Yiangou Y, Mennuni C, Luzzago A, Ludvigsson J, et al. Osteopontin is an autoantigen of the somatostatin cells in human islets: identification by screening random peptide libraries with sera of patients with insulin-dependent diabetes mellitus. Vaccine 1999; 18(3-4):342-54.
34. Ola TO, Biro PA, Hawa MI, Ludvigsson J, Locatelli M, Puglisi MA, et al. Importin beta: a novel autoantigen in human autoimmunity identified by screening random peptide libraries on phage. J Autoimmun 2006; 26(3):197-207.
35. Devendra D, Galloway TS, Horton SJ, Wilkin TJ. Exploring the idiotypes of insulin antibodies as markers for remission in Type 1 diabetes. Diabet Med. 2004; 21(12):1316-24.
36. Devendra D, Franke B, Galloway TS, Horton SJ, Knip M, Wilkin TJ. Distinct idiotypes of insulin autoantibody in autoimmune polyendocrine syndrome type 2 and childhood onset type 1 diabetes. J Clin Endocrinol Metab 2004; 89(10):5266-70.
37. Dromey JA, Weenink SM, Peters GH, Endl J, Tighe PJ, Todd I, et al. Mapping of epitopes for autoantibodies to the type 1 diabetes autoantigen IA-2 by peptide phage display and molecular modeling: overlap of antibody and T cell determinants. J Immunol 2004;172(7):4084-90.
38. Navone R, Lunardi C, Gerli R, Tinazzi E, Peterlana D, Bason C, et al. Identification of tear lipocalin as a novel autoantigen target in Sjogren's syndrome. J Autoimmun 2005; 25(3):229-34.
39. Cha S, Leung PS, Van de Water J, Tsuneyama K, Joplin RE, Ansari AA, et al. Random phage mimotopes recognized by monoclonal antibodies against the pyruvate dehydrogenase complex-E2 (PDC-E2). Proc Natl Acad Sci U S A 1996; 93(20):10949-54.
40. Leung PS, Cha S, Joplin RE, Galperin C, Van de Water J, Ansari AA, et al. Inhibition of PDC-E2 human combinatorial autoantibodies by peptide mimotopes. J Autoimmun 1996; 9(6):785-93.
41. Mackay IR, Davies JM, Rowley MJ. Towards the pathogenesis of autoimmune liver disease. J Autoimmun 1999; 13(1):163-9.
42. Rowley MJ, Scealy M, Whisstock JC, Jois JA, Wijeyewickrema LC, Mackay IR. Prediction of the immunodominant epitope of the pyruvate dehydrogenase complex E2 in primary biliary cirrhosis using phage display. J Immunol 2000; 164(6):3413-9.
43. Ikuno N, Scealy M, Davies JM, Whittingham SF, Omagari K, Mackay IR, et al. A comparative study of antibody expressions in primary biliary cirrhosis and autoimmune cholangitis using phage display. Hepatology. 2001; 34(3):478-86.
44. Scealy M, Mackay IR, Rowley MJ. Amino acids critical for binding of autoantibody to an immunodominant conformational epitope of the pyruvate dehydrogenase complex subunit E2: identification by phage display and site-directed mutagenesis. Mol Immunol 2006; 43(3):745-53.
45. Beland K, Lapierre P, Marceau G, Alvarez F. Anti-LC1 autoantibodies in patients with chronic hepatitis C virus infection. J Autoimmun 2004; 22(2):159-66.
46. Mecchia M, Casato M, Tafi R, Filocamo G, Bonomo L, Fiorilli M, et al. Nonrheumatoid IgM in human hepatitis C virus-associated type II cryoglobulinemia recognize mimotopes of the CD4-like LAG-3 protein. J Immunol 1996;157(8):3727-36.
47. Lunardi C, Bason C, Navone R, Millo E, Damonte G, Corrocher R, Puccetti A. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med 2000; 6(10):1183-6.
48. Jadali Z, Eslami MB, Sanati MH, Mansouri P, Mahmoudi M, Maghsoudi N, et al. Identification of peptides specific for antibodies in vitiligo using a phage library. Clin Exp Dermatol 2005; 30(6):694-701.
49. Jadali1 Z, Eslami MB, Sanati MH, Mansoori P, Mahmoudi M, Maghsoodi N, et al. Identification of Hitherto Undefined B-Cell Epitopes by Antibodies in the Sera of Vitiligo Patients Using Phage-Display Peptide Library. Iran J Allergy Asthma Immunol 2003; 2(4):197-201.
50. Iverson GM, Jones DS, Marquis D, Linnik MD, Victoria EJ. A chemically defined, toleragen-based approach for targeting anti-beta2-glycoprotein I antibodies. Lupus 1998; 7(suppl 2):S166-9.
51. Koike T, Ichikawa K, Kasahara H, Atsumi T, Tsutsumi A, Matsuura E. Epitopes on beta2-GPI recognized by anticardiolipin antibodies. Lupus 1998; 7(Suppl 2):S14-7.
52. Blank M, Shoenfeld Y, Cabilly S, Heldman Y, Fridkin M, Katchalski-Katzir E. Prevention of experimental

antiphospholipid syndrome and endothelial cell activation by synthetic peptides. Proc Natl Acad Sci U S A 1999; 96(9): 5164-8.

53. Visvanathan S, Scott JK, Hwang KK, Banares M, Grossman JM, Merrill JT, et al. Identification and characterization of a peptide mimetic that may detect a species of disease-associated anticardiolipin antibodies in patients with the antiphospholipid syndrome. Arthritis Rheum 2003; 48(3):737-45.
54. Kasahara H, Matsuura E, Kaihara K, Yamamoto D, Kobayashi K, Inagaki J, et al. Antigenic structures recognized by anti-beta2-glycoprotein I auto-antibodies. Int Immunol 2005; 17(12): 1533-42.
55. Fischer C, Buschmann K, Blank M, Stachl A, Miesbach W, Shoenfeld Y, et al. Identification of a peptide mimicking the binding pattern of an antiphospholipid antibody. Immunobiology. 2006; 211(9): 695-9.
56. Byun CH, Park JY, Akamizu T, Chae CB. Identification of the peptides that inhibit the function of human monoclonal thyroid-stimulating antibodies from phage-displayed peptide library. J Clin Endocrinol Metab 2001; 86(7):3311-8.
57. Na CH, Lee MH, Cho BY, Chae CB. A method for identification of the peptides that bind to a clone of thyroid-stimulating antibodies in the serum of Graves' disease patients. J Clin Endocrinol Metab 2003; 88(4):1570-6.
58. Bresson D, Cerutti M, Devauchelle G, Pugniere M, Roquet F, Bes C, et al. Localization of the discontinuous immunodominant region recognized by human anti-thyroperoxidase autoantibodies in autoimmune thyroid diseases. J Biol Chem 2003; 278(11):9560-9.
59. Cook AD, Davies JM, Myers MA, Mackay IR, Rowley MJ. Mimotopes identified by phage display for the monoclonal antibody CII-C1 to type II collagen. J Autoimmun 1998; 11(3): 205-11.
60. Davies JM, Rowley MJ, MacKay IR. Phagotopes derived by antibody screening of phage-displayed random peptide libraries vary in immunoreactivity: studies using an exemplary monoclonal antibody, CII-C1, to type II collagen. Immunol Cell Biol 1999; 77(6): 483-90.
61. Davies JM, Mackay IR, Rowley MJ. Rheumatoid arthritis sera react with a phage-displayed peptide selected by a monoclonal antibody to type II collagen that has homology to EBNA-1. Autoimmunity. 1999; 30: 53-9.
62. Xu Y, Ramsland PA, Davies JM, Scealy M, Nandakumar KS, Holmdahl R, et al. Two monoclonal antibodies to precisely the same epitope of type II collagen select non-crossreactive phage clones by phage display: implications for autoimmunity and molecular mimicry. Mol Immunol 2004; 41(4):411-9.
63. Dybwad A, Forre O, Kjeldsen-Kragh J, Natvig JB, Sioud M. Identification of new B cell epitopes in the sera of rheumatoid arthritis patients using a random nanopeptide phage library. Eur J Immunol 1993; 23(12):3189-93.
64. Dybwad A, Forre O, Natvig JB, Sioud M. Structural characterization of peptides that bind synovial fluid antibodies from RA patients: a novel strategy for identification of disease-related epitopes using a random peptide library. Clin Immunol Immunopathol 1995; 75(1):45-50.
65. Dybwad A, Forre O, Sioud M. Increased serum and synovial fluid antibodies to immunoselected peptides in patients with rheumatoid arthritis. Ann Rheum Dis 1996; 55(7): 437-41.
66. Sioud M, Dybwad A, Jespersen L, Suleyman S, Natvig JB, Forre O. Characterization of naturally occurring autoantibodies against tumour necrosis factor-alpha (TNF-alpha): in vitro function and precise epitope mapping by phage epitope library. Clin Exp Immunol 1994; 98(3):520-5.
67. Matsushita S, Nishi T, Oiso M, Yamaoka K, Yone K, Kanai T, et al. HLA-DQ-binding peptide motifs. 1. Comparative binding analysis of type II collagen-derived peptides to DR and DQ molecules of rheumatoid arthritis-susceptible and non-susceptible haplotypes. Int Immunol 1996; 8(5):757-64.
68. Zhang M, Davidson A. A rheumatoid factor specific mimotope identified by a peptide display library. Autoimmunity 1999; 30(3):131-42.
69. von Landenberg P, Rzepka R, Melchers I. Monoclonal autoantibodies from patients with autoimmune diseases: synovial fluid B lymphocytes of a patient with rheumatoid arthritis produced an IgG lambda antibody recognizing J-sequences of Ig kappa chains in a conformation-dependent way. Immunobiology 1999; 200(2):205-14.
70. von Landenberg P, von Landenberg C, Grundl M, Schmitz G, Scholmerich J, Melchers I. A new antigenic epitope localized within human kappa light chains specific for rheumatoid arthritis and systemic lupus erythematosus. J Autoimmun 1999; 13(1):83-7.
71. Hansen MH, Dybwad A, Forre O, Sioud M. Probing antinuclear antibody specificities by peptide phage display libraries. Clin Exp Rheumatol 2000; 18(4):465-72.
72. Lee L, Buckley C, Blades MC, Panayi G, George AJ, Pitzalis C. Identification of synovium-specific homing

peptides by in vivo phage display selection. Arthritis Rheum 2002; 46(8):2109-20.
73. Cortese I, Tafi R, Grimaldi LM, Martino G, Nicosia A, Cortese R. Identification of peptides specific for cerebrospinal fluid antibodies in multiple sclerosis by using phage libraries. Proc Natl Acad Sci U S A 1996; 93(20):11063-7.
74. Dybwad A, Flrre O, Sioud M. Probing for cerebrospinal fluid antibody specificities by a panel of random peptide libraries. Autoimmunity 1997; 25(2):85-9.
75. Cortese I, Capone S, Tafi R, Grimaldi LM, Nicosia A, Cortese R. Identification of peptides binding to IgG in the CSF of multiple sclerosis patients. Mult Scler 1998; 4(1):31-6.
76. Cortese I, Capone S, Luchetti S, Grimaldi LM, Nicosia A, Cortese R. CSF-enriched antibodies do not share specificities among MS patients. Mult Scler 1998; 4(3):118-23.
77. Rand KH, Houck H, Denslow ND, Heilman KM. Molecular approach to find target(s) for oligoclonal bands in multiple sclerosis. J Neurol Neurosurg Psychiatry 1998; 65(1):48-55.
78. Rand KH, Houck H. Improved methods for the application of random peptide phage libraries to the study of the oligoclonal bands in cerebrospinal fluid of patients with multiple sclerosis. J Neurosci Methods 2000; 101(2):131-9.
79. Cortese I, Capone S, Luchetti S, Cortese R, Nicosia A. Cross-reactive phage-displayed mimotopes lead to the discovery of mimicry between HSV-1 and a brain-specific protein. J Neuroimmunol 2001;113(1):119-28.
80. Jolivet-Reynaud C, Perron H, Ferrante P, Becquart L, Dalbon P, Mandrand B. Specificities of multiple sclerosis cerebrospinal fluid and serum antibodies against mimotopes. Clin Immunol 1999; 93(3):283-93.
81. Yu X, Gilden DH, Ritchie AM, Burgoon MP, Keays KM, Owens GP. Specificity of recombinant antibodies generated from multiple sclerosis cerebrospinal fluid probed with a random peptide library. J Neuroimmunol. 2006; 172(1-2):121-31.
82. Yu X, Owens GP, Gilden DH. Rapid and efficient identification of epitopes/mimotopes from random peptide libraries. J Immunol Methods 2006; 316(1-2):67-74.
83. Sibille P, Ternynck T, Nato F, Buttin G, Strosberg D, Avrameas A. Mimotopes of polyreactive anti-DNA antibodies identified using phage-display peptide libraries. Eur J Immunol 1997; 27(5):1221-8.
84. Gaynor B, Putterman C, Valadon P, Spatz L, Scharff MD, Diamond B. Peptide inhibition of glomerular deposition of an anti-DNA antibody. Proc Natl Acad Sci U S A 1997; 94(5):1955-60.
85. Putterman C, Diamond B. Immunization with a peptide surrogate for double-stranded DNA (dsDNA) induces autoantibody production and renal immunoglobulin deposition. J Exp Med 1998; 188(1):29-38.
86. Putterman C, Deocharan B, Diamond B. Molecular analysis of the autoantibody response in peptide-induced autoimmunity. J Immunol 2000; 164(5):2542-9.
87. Beger E, Deocharan B, Edelman M, Erblich B, Gu Y, Putterman C. A peptide DNA surrogate accelerates autoimmune manifestations and nephritis in lupus-prone mice. J Immunol 2002; 168(7):3617-26.
88. Sharma A, Isenberg D, Diamond B. Studies of human polyclonal and monoclonal antibodies binding to lupus autoantigens and cross-reactive antigens. Rheumatology 2003; 42(3):453-63.
89. Sun Y, Fong KY, Chung MC, Yao ZJ. Peptide mimicking antigenic and immunogenic epitope of double-stranded DNA in systemic lupus erythematosus. Int Immunol 2001; 13(2):223-32.
90. Dieker JW, Sun YJ, Jacobs CW, Putterman C, Monestier M, Muller S, et al. Mimotopes for lupus-derived anti-DNA and nucleosome-specific autoantibodies selected from random peptide phage display libraries: facts and follies. J Immunol Methods 2005; 296(1-2):83-93.
91. Zhang W, Reichlin M. A peptide DNA surrogate that binds and inhibits anti-dsDNA antibodies. Clin Immunol 2005; 117(3):214-20.
92. Martin F, Chan AC. Pathogenic roles of B cells in human autoimmunity; insights from the clinic. Immunity 2004; 20(5):517-27.
93. Silverman GJ, Carson DA. Roles of B cells in rheumatoid arthritis. Arthritis Res Ther 2003; 5(Suppl 4):S1-6.
94. Ruff RL, Lennon VA. End-plate voltage-gated sodium channels are lost in clinical and experimental myasthenia gravis. Ann Neurol 1998; 43(3):370-9.
95. Deroo S, Muller CP. Antigenic and immunogenic phage displayed mimotopes as substitute antigens: applications and limitations. Comb Chem High Throughput Screen 2001; 4(1):75-110.