
The presence of autoantibodies is the hallmark of systemic autoimmune diseases. Although their pathogenetic and aetiological relationship is not fully understood, autoantibodies are important tools for establishing the diagnosis, classification and prognosis of autoimmune diseases. Autoantibodies in systemic autoimmune rheumatic diseases target mainly multicomponent intracellular complexes containing both protein antigens and (ribo)-nucleic acid(s), such as the spliceosome or Ro/La RNPs. In this article we focus on their B-cell antigenic determinants (epitopes). We first provide a brief overview of the types of B-cell epitope that have been identified. They include primary, secondary, tertiary and quaternary structured epitopes, as well as cryptotopes, neoepitopes and mimotopes. New insights into the pathogenetic role of epitopes in systemic autoimmunity are also examined. In this regard, using the defined structures of the B-cell antigenic epitopes, complementary epitopes can be designed according to the ‘molecular recognition’ theory. The complementary epitopes can be used as probes to study pathogenetic and immunoregulatory aspects of the anti-idiotypic response. The origin of humoral autoimmunity and the spreading of the epitopes during the evolution of the autoimmune response are also discussed. Finally, the ability of post-translational modifications to induce autoreactive immune attack via the generation of neoepitopes is presented.
Epitopes of Autoantigens and their Role in Initiation and Propagation of Systemic Autoimmunity

John G Routsiaras and Athanasios G Tzioufas

Department of Pathophysiology, School of Medicine, Athens

Abstract
The presence of autoantibodies is the hallmark of systemic autoimmune diseases. Although their pathogenetic and aetiological relationship is not fully understood, autoantibodies are important tools for establishing the diagnosis, classification and prognosis of autoimmune diseases. Autoantibodies in systemic autoimmune rheumatic diseases target mainly multicomponent intracellular complexes containing both protein antigens and (ribo)-nucleic acid(s), such as the spliceosome or Ro/La RNPs. In this article we focus on their B-cell antigenic determinants (epitopes). We first provide a brief overview of the types of B-cell epitope that have been identified. They include primary, secondary, tertiary and quarternary structured epitopes, as well as cryptotopes, neoepitopes and mimotopes. New insights into the pathogenetic role of epitopes in systemic autoimmunity are also examined. In this regard, using the defined structures of the B-cell antigenic epitopes, complementary epitopes can be designed according to the 'molecular recognition' theory. The complementary epitopes can be used as probes to study pathogenetic and immunoregulatory aspects of the anti-idiotypic response. The origin of humoral autoimmunity and the spreading of the epitopes during the evolution of the autoimmune response are also discussed. Finally, the ability of post-translational modifications to induce autoreactive immune attack via the generation of neoepitopes is presented.

Keywords
Autoimmunity, rheumatic diseases, B-cell epitopes, La/SSB, Ro/SSA

Disclosure: The authors have no conflicts of interest to declare.

Received: 29 July 2009 Accepted: 16 August 2009

Correspondence: Athanasios G Tzioufas, Department of Pathophysiology, School of Medicine, 75, M Asias St, 11527, Athens, Greece. E: agtzi@med.uoa.gr

Historically, studies of disease mechanisms in systemic autoimmunity have focused on characterisation of autoantibodies and identification of their antigenic targets. The understanding of fine autoantibody specificities in systemic autoimmune rheumatic diseases is important in both the basic and clinical settings. In fact, many autoantibodies to intracellular autoantigens are essential for the evaluation of patients with systemic rheumatic diseases: they are included in the diagnostic or classification criteria of certain systemic autoimmune disorders; some are associated with disease activity indices, particularly in systemic lupus erythematosus (SLE); and some might be correlated with specific clinical manifestations observed in the spectrum of a given systemic disease (i.e. anti-Ro/SSA antibodies with skin involvement in subacute cutaneous lupus erythematosus [SCLE]). In basic research focusing on autoimmune diseases, the most exciting question remains unaddressed: why are these particular autoantigens, among the many molecules expressed in the organism, selected as targets of the immune system? The answer to this question is certainly a significant step towards our understanding of the origin of autoimmunity. Over the past few years, different laboratories have tried to define the fine specificity of autoantibodies to intracellular antigens by identifying those structures within the antigen moiety recognised most frequently by autoantibodies (antigenic determinants or B-cell epitopes). The identification of B-cell epitopes may provide useful information on putative mechanisms regarding the generation of autoantibodies, including molecular mimicry (molecular similarity that leads to cross-reactivity between antigens from a foreign agent and self-proteins) and epitope spreading (expansion of an autoantibody’s target from a single determinant to a number of distinct and non-cross-reactive epitopes on a given autoantigen). In addition, characterisation of epitopes with high sensitivity and specificity may facilitate the development of immunoassays based on synthetic peptides that eventually can be used as substrates for the detection of autoantibodies. Finally, the association of a specific epitope with the clinical picture or certain clinical findings may be proved valuable for potential therapeutic applications, including the generation of peptide-based vaccines.

Classification and Characteristics of B-cell Epitopes of Autoantigens
B-cell epitopes are diverse in structure and immune reactivity and can be classified accordingly. On the basis of epitope nature, they can be classified as linear or continuous, consisting of sequential amino acids in the primary structure of the protein, and conformational or discontinuous epitopes, formed by distant regions in the protein sequence coming together in its secondary, tertiary or quarternary structure (in the case of ribonucleoprotein complexes). Additional types include cryptic epitopes (cryptotopes). These are usually linear epitopes hidden in the native structure of the autoantigen. They become accessible to antibody binding after disruption of the 3D structure (e.g. by denaturation, proteolytic
Autoimmune Diseases Rheumatoid Arthritis

degradation or chemical modification of the autoantigen). Modified epitopes (neoepitopes) incorporate post-translationally modified amino acids such as phosphorylated serine, threonine or tyrosine, acetylated lysine, deamidated glutamine, glycosylated asparagine, serine or threonine and citrullinated arginine in their structure. Such modified amino acids have been reported in a variety of human nuclear proteins, including the Sm antigens D1 and D3, fibrillarin and nucleolin. B-cell Epitopes of Autoantigens as Tools to Study the Autoimmune Response Complementary Epitopes and Anti-idiotypic Antibodies

The idiopathic network theory was proposed by the 1984 Nobel laureate Niels Jerne. He hypothesised that antibodies can act as antigens and elicit antiantibodies (called anti-idiotypic antibodies). If anti-idiotypic antibodies target the antigen-binding sites of the idiotype antibodies, they can either compete with the antigen for the same binding site (Ab2 anti-idiotypic antibodies according to Jerne’s classification) or elicit anti-idiotype antibodies with similar antigenic specificity to the idiotype antibodies. Thus, anti-idiotype antibodies can either neutralise idiotype antibodies or elicit the generation of antibodies with the original antigenic specificity. In this regard, after an antigenic stimulus, an anti-idiotypic network is established regulating the production of antibodies directed against the antigen that initiated the immune response.

Based on the detailed knowledge of the antigenic structures that are recognised by autoantibodies, one can design complementary epitopes that are anticipated to be recognised by anti-idiotypic antibodies, according to the ‘molecular recognition’ theory. Molecular recognition theory supports that a sense peptide (transcribed and translated from a nucleotide sequence read in the 5’-→3’-direction) binds to its complementary peptide counterpart, and is transcribed and translated in frame with that of its sense peptide from a nucleotide sequence read in the 5’-→3’-direction on the opposite DNA strand. Many experimental data suggest that these interacting peptides have the ability to generate and detect interacting pairs of idiotypic and anti-idiotype antibodies. Recent findings indicate that autoimmunity can be initiated through an immune response against a peptide that is complementary to the idiotypic antibodies, they can either compete with the antigen for the same binding site (Ab2 anti-idiotypic antibodies according to Jerne’s classification) or elicit anti-idiotype antibodies with similar antigenic specificity to the idiotype antibodies. Thus, anti-idiotype antibodies can either neutralise idiotype antibodies or elicit the generation of antibodies with the original antigenic specificity. In this regard, after an antigenic stimulus, an anti-idiotypic network is established regulating the production of antibodies directed against the antigen that initiated the immune response.

The role of the idiotype-anti-idiotype network was evaluated in the development of neonatal lupus syndrome (NLS) and particularly its most important manifestation, congenital heart block (CHB), in pregnant women with anti-Ro/SSA and/or anti-La/SSB antibodies. Among the systemic autoimmune diseases, NLS is the ideal model for studying anti-idiotypic antibodies, since pathogenetic autoantibodies to Ro/SSA and/or La/SSB are directly involved in tissue injury. When the autoantibodies are binding onto the developing myocardium of the foetuses, CHB is produced. We studied the idiotypic/anti-idiotypic network of antibodies targeting the dominant epitopes of La/SSB in mothers positive for anti-Ro and/or anti-La/SSB antibodies. It was found that sera from mothers giving birth to a healthy child and having no history of a child with neonatal lupus syndrome–CHB (NLS-CHB) exhibited higher anti-idiotype antibody activity compared with the group of mothers carrying a child with NLS-CHB or mothers giving birth to a healthy child but who previously gave birth to a child with NLS. Sera from mothers of healthy children who exhibited no apparent epitope activity against amino acids 349–364 revealed a significantly higher frequency of hidden anti-349–364aa epitope responses blocked by anti-idiotype antibodies compared with sera from women pregnant with an affected child. It seems that the presence of anti-idiotype antibodies to autoantibodies against La/SSB may protect the foetus by blocking pathogenic maternal autoantibodies (see Figure 1).

Testing for these anti-idiotypic responses may be useful in predicting a decreased risk of NLS.

Early Epitope Recognition in Autoimmune Diseases and Epitope Spreading

The initiation of autoimmunity occurs when the tolerance to self-antigens is broken, a phenomenon that has fascinated immunologists for over a century. During the past few decades, innovative methodologies for screening and analysing cellular and biochemical processes have led to an extensive body of experimental data that characterises human autoimmune diseases on multiple levels. Nevertheless, the precise aetiology of most human autoimmune diseases remains largely unexplained, and the immunogens initiating the autoimmune cascade are still not well understood.
understood. The onset and progression of autoantibody development before clinical diagnosis has been studied in SLE. Using the US Department of Defense Serum Repository, with over 30 million specimens prospectively collected from 5 million US Armed Forces personnel, Arbuckle et al. evaluated serum samples obtained from 130 persons before they received a diagnosis of SLE.23 They found that in 115 of the 130 patients with SLE (88%), at least one SLE autoantibody tested was present before the diagnosis (up to 9.4 years earlier; mean 3.3 years). ANA, anti-phospholipid, anti-Ro and anti-La antibodies were present earlier than anti-Smith antigen (anti-Sm) and anti-US small nuclear ribonucleoprotein (anti-snRNP) antibodies. Anti-dsDNA antibodies were found later than ANA and earlier than anti-snRNP antibodies. The earliest autoantibodies detected in the pre-clinical period as individuals progressed towards clinical SLE were antibodies directed to Ro60 protein, a protein component of Ro autoantigen (mean 3.7 years before disease onset). McClain et al. mapped the initial pre-disease target of the anti-Ro60 autoantibody response to the region, 169–180aa (TKYQRNGWSHK), of the autoantigen.13 This region belongs to the SLE-related 169–190aa epitope previously identified by Routtias et al.14 McClain and co-workers disclosed that epitope 169–180aa cross-reacts with a peptide (GGSGSGPRHRDGVRR, 58–72aa) from the latent viral protein Epstein-Barr virus nuclear antigen-1 (EBNA-1).20 Notably, no areas of primary sequence homology exist between EBNA-1 58–72aa and Ro169–180aa, although both of these peptides have similarly high isoelectric points (12.0 and 10.5, respectively). However, animals immunised with either the 169–180 epitope of the cross-reactive EBNA-1 epitope progressively developed autoantibodies binding multiple epitopes of Ro and spliceosomal autoantigens.25 These animals eventually developed some of the clinical symptoms of lupus, such as leukocytopenia, thrombocytopenia and renal dysfunction. Although these experiments indicate a cross-reaction between the initial epitope of Ro60 and the S8–72aa region of EBNA-1, any involvement of Epstein Barr virus in the pathogenesis of SLE has to be elucidated.

After the initial response against Ro60 autoantigen, autoantibody targets can be expanded to the whole Ro60 by a procedure known as epitope spreading. The term epitope spreading was introduced in the early 1990s to describe the ability of the B- and T-cell immune response to diversify, at the level of specificity, from a single determinant to many different sites of an autoantigen (see Figure 2).22 This process is not a feature restricted to systemic autoimmune diseases but is a common characteristic of the natural immune response mounted against several pathogens. In this regard, McClain et al. showed that immunisation of rabbits with an antigenic peptide of Ro60 autoantigen (274–289aa) led to antibodies to multiple epitopes of Ro60 and La. In addition, cross-reactive antibodies to the common spliceosomal proteins Sm B’, Sm D1, RNP A and RNP C were produced.23 These results demonstrate that loss of tolerance to a single antigenic determinant of the autoantigen can begin an autoimmune response that virtually recreates the humoral autoimmune specificity seen in human SLE. Clues to the mechanisms involved in the aforementioned production of cross-reactive antibodies to the common spliceosomal proteins have been reported by Monneau et al.22,23 According to their model, a consensus sequence (the RNP motif) conserved in many nuclear, nucleolar and cytoplasmic antigens plays the role of a ‘driver’ epitope. Cross-reactive autoantibodies targeting this epitope have the potential to spread the autoimmune response to other RNA-binding proteins through molecular mimicry. Subsequently, intramolecular spreading to these specific proteins can occur. This hypothesis is based on the observation that this driver epitope sequence in the RNP motif is recognised by CD4+ T cells from lupus mice and is often targeted by autoantibodies very early during the course of the disease.22,24 Remarkably, this sequence is present in components of Ro/La RNP such as Ro60 (119–131aa), La (146–158aa) and nucleolin (346–358aa, 517–529aa), as well as in spliceosomal proteins such as RNP 70 (139–151aa) and RNP A (47–59aa, 239–251aa). Several other sequences might be considered as important ‘initiator’ sequences, e.g. the recurring proline-rich sequence PGPMPP, present in several snRNPs and recently identified as early target of RNP humoral autoimmunity in SLE,25 or the dimethylarginine-modified CRG repeats present on the B, D1 and D3 autoantigens.26

**Figure 1: Proposed Role of Anti-idiotypic Antibodies in Autoimmune Congenital Heart Block**

A: Mothers pregnant with anti-Ro/anti-La antibodies and healthy children contain in their sera anti-idiotypic antibodies. Anti-idiotypic antibodies bind to and neutralise the antibodies to the major epitope of La/SSB and protect the foetus from the pathogenic effects of the latter. B: in the absence of anti-idiotypic antibodies, maternal anti-Ro/SSA and/or anti-La/SSB antibodies cross the placenta and opsonise foetal apoptotic cardiomyocytes, which in turn induce a pro-inflammatory/pro-fibrotic response, ultimately leading to tissue injury.

**Figure 2: Epitope Spreading**

One CD4+ T-cell clone recognising an epitope on La/SSB autoantigen can activate numerous La-specific and Ro-specific B-cell clones targeting different epitopes within the two autoantigens.
Autoimmune Diseases  

The majority of mammalian proteins have post-translational modifications (PTMs) that can potentially be recognised by the immune system as self newly constructed epitopes (neoepitopes). PTMs are usually catalysed by enzymes but they can also occur spontaneously. Two amino acid modifications have been described as targets of systemic autoimmunity (see Figure 3).

**Arginine Modifications**

Arginine residues are susceptible to three forms of modification: methylation, deimination and citrullination. Two of them have been correlated with systemic autoimmunity (see Figure 3).

**Dimethylation**

The Sm proteins D1, D3 and B/B’ contain a C-terminal rich in arginine and glycine residues that is conserved in most eukaryotic organisms. Studies using mass spectrometry and sequencing of the C-terminus of these Sm proteins have shown that repeated RG dipeptide regions in Sm D1 and Sm D3 and repeated GRG triplets in Sm B/B’ contain symmetrical dimethylarginine residues. Dimethylation of arginine residues of the major Sm D1 and Sm D3 autoepitopes has been reported to remarkably increase binding by SLE autoantibodies. Moreover, a particular Sm D3 peptide represents a highly specific substrate for detecting a subclass of anti-Sm antibodies by enzyme-linked immunosorbent assay (ELISA). Thus, symmetrical dimethylarginine acts as targets for autoantibodies in SLE. It was recently shown that the same autoantigens contain, in addition to the already-reported symmetrical dimethylarginine, asymmetrical dimethylarginine. The effect of this modification in autoantibody binding has not been studied yet.

**Citrullination**

Removal of the imine group from an arginine residue produces citrulline, which lacks the positive charge of arginine. This reaction is catalysed by the peptidylarginine deiminase (PAD) family of enzymes. Citrulline has recently attracted interest as an autoantibody target in rheumatoid arthritis (RA). One of the major autoantigens in RA, filaggrin, is citrullinated by PAD and provides several targets for autoantibody binding. Using a selected set of citrullinated peptides, Schellekens et al. developed an ELISA with a sensitivity of more than 70% and an impressive specificity of more than 96%. The same investigators developed a new diagnostic test for sera using a cyclic citrullinated peptide (CCP). Using RA and non-RA sera, the anti-CCP ELISA proved to be highly specific (98%) with reasonable sensitivity (68%). Compared with the immunoglobulin M rheumatoid factor (IgM-RF) ELISA, the anti-CCP ELISA possessed a significantly higher specificity (96% for CCP versus 91% for IgM-RF). The sensitivity of both tests for RA was moderate: 48 and 54% for the anti-CCP ELISA and the IgM-RF ELISA, respectively. The combination of anti-CCP and IgM-RF results in a significantly higher positive predictive value of 91% and a slightly lower negative predictive value of 78% than the IgM-RF ELISA alone. Similarly, when anti-CCP and RF antibodies were combined, the specificity exceeded 99%. Finally, follow-up studies revealed that anti-CCP antibodies could be detected in early phases of RA and that these antibodies were associated with more severe radiological damage.
Epitopes of Autoantigens and their Role in Initiation and Propagation of Systemic Autoimmunity

Serine/Threonine Phosphorylation
Phosphorylation is the most common and ubiquitous form of enzyme-mediated PTM. It has been implicated in the recognition of nuclear autoantigens by the immune system in SLE.

Spliceosomal Antigens
Phosphorylated components of U1 snRNP particles are specifically recognised by autoantibodies of SLE patients and CD4+ T cells from lupus-prone mice (MRL/lpr mice). Monneau et al. demonstrated the importance of phosphorylation of a specific serine residue of U1-70K in MRL/lpr mice. Previously, these investigators had identified an epitope at residues 131–150 of the U1-70K protein that was recognised by antibodies as well as by CD4+ T cells in two strains of lupus mice. They subsequently synthesised two peptides, one with a phosphate group on serine residue 137 and the other with a phosphate group on serine residue 140. It was demonstrated that the peptide with Ser140P but not the peptide with Ser137P was recognised by both antibodies and CD4+ T cells.

La Autoantigen
La protein can be phosphorylated at position 366. La phosphorylated at serine 366 is nucleoplasmic and is associated with nascent RNA polymerase III transcripts, while non-phosphorylated La is cytoplasmic and is associated with a subset of mRNAs that contain polymerase III transcripts, while non-phosphorylated La is nucleoplasmic and is associated with nascent RNA that differ in subcellular localisation and is associated RNAs, which can be discerned by serine 366 phosphorylation. This specific phosphorylation resides within the major B-cell epitope that was previously identified in our laboratory to be located in the 349–368aa region of La/Ssb. Our studies indicate that the antigenicity of the epitope is significantly enhanced upon phosphorylation of serine 366.

Future Directions
The extensive study of B-cell epitopes of intracellular autoantigens provides useful insights into the diagnosis, classification and prognosis of autoimmune diseases. The successful development of diagnostic assays is hindered by a number of factors concerning epitope recognition in autoimmune disorders, such as cross-reactivity, epitope spreading, epitope masking and epitope modification. Issues regarding the simultaneous analysis of a large number of autoantibody specificities in a single test also have to be considered. The analysis of B-cell epitopes of autoantigens provides clues to overcome these problems. Autoantibody screening test methodologies can be improved using large-scale arrays with specific autoantigen epitopes. These arrays are able to perform large-scale multiplex characterisations of autoantibody responses against structurally diverse autoantigens. Chemical modifications of epitopes of autoantigens can provide better antigenic substrates mimicking naturally occurring PTMs. The lesson of citrullinated peptides taught us that recombinant proteins are not always the preferred substrate for autoantibody detection and that synthetic peptides can be successfully used in diagnostic assays if the exact structure of the autoantibody target is known. Complementary peptides can efficiently neutralise anti-idiotypic antibodies, enhancing the interaction of the idiotypic autoantibodies with their target epitope.

The analysis of B-cell epitopes of autoantigens provides a better understanding of the origin and evolution of autoimmunity response. In this regard, epitopes from foreign antigens, mimicking complementary epitopes or post-translationally modified peptides, could be the initiating agents of autoimmune disease. In addition, the spreading of autoimmune response from the initiating epitope others can be utilised for monitoring the evolution of autoimmune disease. Finally, the analysis of B-cell epitopes of autoantigens can provide potential therapeutic regimens, using epitopes with high specificity as vaccines, as tolerogens or as modifiers of the autoimmune response via the idiotypic–anti-idiotypic network.

Conclusion
Autoantibodies to intracellular autoantigens remain a significant laboratory tool to evaluate patients with rheumatic autoimmune diseases. The identification of their B-cell epitopes provides new ways of understanding pathogenetic aspects in systemic autoimmunity, including epitope spreading, idiotypic-anti-idiotypic antibody interaction and neoepitope formation due to PTMs of autoantigens. Finally, some B-cell epitopes appear to be promising diagnostic markers for the evaluation of systemic autoimmune disorders.