Routsias, J. G., P. G. Vlachoyiannopoulos, and A. G. Tzioufas. 2006. **Αυτοαντισώματα έναντι ενδοκυττάριων αυτοαντιγόνων και οι Β-κυτταρικοί επιτοποι τους: μοριακά εργαλεία για την μελέτη της αυτοάνοσης απόκρισης**. Critical reviews in clinical laboratory sciences 43:203-248.

A common laboratory finding in systemic autoimmune diseases is the presence of autoantibodies against intracellular autoantigens. Although their pathogenesis is not fully understood, autoantibodies are important tools for establishing diagnosis, classification, and prognosis of autoimmune diseases. Autoantibodies mainly target multicomponent complexes containing both protein antigens and (ribo)-nucleic acid(s), such as the spliceosome or Ro/La RNPs. In this review, we address the main characteristics and the clinical value of the main autoantibody types with respect to their disease association, and we describe the corresponding autoantigens, their biologic function, and their B-cell antigenic determinants (epitopes). The structural characteristics and clinical associations of these epitopes, and their utility as tools to investigate the autoimmune response, are discussed in detail. 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. These 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 in systemic lupus erythematosus are also discussed. Finally, the ability of post-translational modifications to induce autoreactive immune attack via the generation of neo-epitopes is summarized.

Critical Reviews in Clinical Laboratory Sciences, 43(3):203–248 (2006)

ISSN: 1040-8363 print / 1549-781X online
DOI: 10.1080/10408360500523837

AUTOANTIBODIES TO INTRACELLULAR AUTOANTIGENS AND THEIR B-CELL EPITOPES: Molecular Probes to Study the Autoimmune Response

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□ *A common laboratory finding in systemic autoimmune diseases is the presence of autoantibodies against intracellular autoantigens. Although their pathogenesis is not fully understood, autoantibodies are important tools for establishing diagnosis, classification, and prognosis of autoimmune diseases. Autoantibodies mainly target multicomponent complexes containing both protein antigens and (ribo)-nucleic acid(s), such as the spliceosome or Ro/La RNPs. In this review, we address the main characteristics and the clinical value of the main autoantibody types with respect to their disease association, and we describe the corresponding autoantigens, their biologic function, and their B-cell antigenic determinants (epitopes). The structural characteristics and clinical associations of these epitopes, and their utility as tools to investigate the autoimmune response, are discussed in detail. 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. These 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 in systemic lupus erythematosus are also discussed. Finally, the ability of post-translational modifications to induce autoreactive immune attack via the generation of neo-epitopes is summarized.*

Keywords Intracellular autoantigens, B-cell epitopes, rheumatic diseases, autoantibodies, anti-La/SSB, complementary epitopes.

Abbreviations **aa**, amino acids; **ACA**, anti-centromere antibodies; **ACR**, American College of Rheumatology; **AKA**, anti-keratin antibodies; **ANA**, antinuclear antibodies; **ANCA**, anti-neutrophilic cytoplasmic antibodies; **APF**, anti-perinuclear factor; **aPL**, anti-phospholipid; **APS**, anti-phospholipid syndrome; **ATP**, adenosine triphosphate; **β2GPI**, β2 glycoprotein I; **Bip**, GRP78/Bip chaperone; **CCP**, cyclic citrullinated peptide; **CREST**, systemic sclerosis characterized by calcinosis, Raynaud's phenomenon, esophagus

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dysmotility, sclerodactyly, and telangiectasia; **CTL/NK**, cytotoxic T-lymphocyte/natural killer cell; **CNS**, central nervous system; **DM**, dermatomyositis; **dsDNA**, double-stranded DNA; **ELISA**, enzyme-linked immunosorbent assay; **hY RNA**, human cytoplasmic RNA; **IBM**, inclusion body myositis; **IRES**, internal ribosome entry site; **LE**, lupus erythematosus; **MCTD**, mixed connective tissue disease; **MPO**, myeloperoxidase; **NLE**, neonatal lupus erythematosus; **PAD**, peptidylarginine deiminase; **PM**, polymyositis; **PR3**, proteinase 3; **PTM**, post-translational modification; **RA**, rheumatoid arthritis; **RF**, rheumatoid factor; **RNP**, ribonucleoprotein; **RRM**, RNA recognition motif; **SCLE**, subacute cutaneous lupus; **SLE**, systemic lupus erythrematosus; **snRNP**, small nuclear ribonucleoprotein particles; **SRP**, signal recognition pattern; **SS**, Sjogren's syndrome; **SSc**, systemic sclerosis; **tRNA**, transfer RNA; **VDJ recombination**, V, D, and J gene segment assembly which generates rearranged V genes encoding variable (V) region for lymphocyte antigen-receptors; **VSV**, vesicular stomatitis virus.

I. INTRODUCTION

Patients with systemic rheumatic diseases often develop autoantibodies directed against large intracellular complexes composed of a number of proteins that are non-covalently associated with nucleic acid components. From an historic perspective, the era of autoantibody research began with the discovery of lupus erythematosus (LE) cells by Hargraves and colleagues in 1948.[1] These investigators observed these cells in the bone marrow of patients with acute systemic lupus erythematosus (SLE) and postulated that these cells *"... were the result of phagocytosis of free nuclear material. ... "* Later, Friou applied the technique of indirect immunofluorescence and demonstrated the presence of antinuclear antibodies (ANA) in sera of patients with SLE.[2] Almost ten years after the description of LE cells, investigators realized that ANA were a potentially heterogenous population, containing multiple autoantibodies against deoxyribonucleic acid (DNA) and other ribonucleoproteins (RNPs).[3,4]

The characterization and thorough understanding of autoantibody specificities in systemic autoimmune rheumatic diseases are important in both the basic and clinical settings. In the clinical area, many autoantibodies to intracellular autoantigens are essential for the evaluation of patients with systemic rheumatic diseases, as attested by: (i) their inclusion in the diagnostic or classification criteria of certain systemic autoimmune disorders;[5,6] (ii) their association with disease activity indices, particularly in SLE;[7] and (iii) the correlation of some autoantibodies with specific clinical manifestations in the spectrum of a given systemic disease.[8] In this regard, assays for autoantibody determination should be reliable, reproducible, easy to perform, and available in everyday clinical practice.

In basic research focusing on autoimmune diseases, the most intriguing question is why these particular autoantigens, among myriads of molecules expressed in the organism, are selected as targets of the immune system.[9] The answer to this question will certainly contribute significantly to our understanding of the origin of autoimmunity.

Over the past decade, several investigators have tried to define the fine specificity of autoantibodies to intracellular antigens by identifying within the antigen moiety the antigenic determinants (or B-cell epitopes) recognized most frequently by autoantibodies. The identification of B-cell epitopes may provide useful information on putative mechanisms of autoantibody production, such as 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 many sites on an autoantigen).[9] In addition, characterization of autoantigen epitopes with high sensitivity and specificity may facilitate the development of immunoassays based on synthetic peptides that can be utilized as substrates for autoantibody detection. When used as antigenic substrates in diagnostic assays, synthetic peptides have several advantages over recombinant antigens. In contrast to *in vivo* production of recombinant proteins, peptide synthesis is a controlled chemical process that leads to high purity and homogenous, stable antigen preparations.[10] This allows the development of highly-sensitive, specific, and reproducible assays. Such test systems can be useful for defining subgroups of a disease and may offer important information about disease prognosis.[11] Furthermore, clinically relevant peptides have been proposed as potentially useful in the treatment of autoimmune diseases via the use of immobilized peptides to remove pathogenic autoantibodies or as vaccine components.[12,13]

The description and characterization of B-cell epitopes of autoantigens have generated many interesting questions. Do the autoimmune epitopes constitute a few dominant sequences or do they represent multiple disparate regions on an autoantigen? Are the frequency and significance of spreading (recruitment) of the epitopes important for maintenance and perpetuation of the autoimmune response? Are the molecular structures of epitopes suitable to provide support for the hypothesis of epitope mimicry as a trigger for autoimmunity? In this review, we will discuss the clinical and biological relevance of autoantibodies in systemic rheumatic diseases, the major B-cell epitopes of intracellular autoantigens, and recent advances in our understanding of the pathogenesis of systemic autoimmune diseases based on studies of B-cell epitopes.

II. AUTOANTIBODIES AND SYSTEMIC DISEASE

A. Systemic Lupus Erythematosus

SLE is considered the prototypic systemic autoimmune disease, since it is mediated by pathogenic autoantibodies that form immune complexes, activate complement, and eventually lead to tissue damage. The disease can affect the skin, joints, kidneys, lungs, nervous system, serous membranes, and

virtually every organ in the body.[14] It is typically a disease of young women, with a female:male ratio of 9:1. The prevalence of SLE in the general population is 40 to 50 cases per 100,000 persons, although differences between geographic regions exist. SLE appears to be more prevalent in African-Americans and Asians than in Caucasians.[15] The etiology of SLE is still unknown. The clinical course of SLE varies and is characterized by periods of remissions and chronic or acute relapses. The most common pattern is a combination of constitutional symptoms with one or more of the following: skin rashes; arthritis; mouth ulcers; serositis; muscle involvement; vasculitis; glomerulonephritis; and musculoskeletal, hematologic, and central or peripheral nervous involvement.[14] The diagnosis of the disease is based on criteria that have been established and revised by the American College of Rheumatology (ACR).[5,16] A patient fullfils the diagnosis of SLE if four of the 11 ACR criteria are met. Importantly, two of these criteria refer to autoantibody assessment. The autoantibody specificities usually detected in SLE are summarized in Table 1.

The determination of ANA in Hep-2 cell lines by indirect immunofluorescence is the screening test of choice in patients suspected of having SLE.[17] ANA remains a semi-quantitative test and is usually positive in high titer (>1:160) in virtually all patients with SLE. ANA are also present in a variety of other autoimmune disorders including Sjogren's syndrome (SS), scleroderma (SSc), and rheumatoid arthritis (RA). Low titers of ANA are found in sera of 5 to 10% of normal individuals, particularly those older than 65 years.[18,19]

TABLE 1 Common Autoantibodies in Various Clinical Forms of Lupus

Disease	Autoantibody target	Frequency (%)
Systemic lupus erythematosus (SLE)	ANA*	90–95
	dsDNA*	65–75
	RF	15–35
	Sm*	20
	RNP	25–30
	Ro/SSA	30–40
	La/SSB	10–15
	Ribosomal P	10–20
	Phospholipid*	30–50
	Ku	10
	Calreticulin	35
Subacute cutaneous	ANA	70
LE (SCLE)	Ro/SSA	>80
Neonatal lupus erythematosus (NLE)	ANA	30
	Ro/SSA	100
	La/SSB	60
Drug induced LE	ANA	>90
	Histone	95–100

*Included in the ACR criteria for the diagnosis of SLE.

Anti-double-stranded DNA (dsDNA) antibodies are highly specific (97%) and virtually diagnostic for SLE, being observed in approximately 65 to 75% of patients with the disease.[20] The titer of anti-dsDNA antibodies is also clinically important in terms of disease activity. Indeed, anti-dsDNA titers rise when the disease is active and usually fall when the flare subsides. A high titer of anti-dsDNA antibodies may indicate severe organ involvement, and, more frequently, concomitant lupus nephritis.[21,22]

The presence of anti-Sm antibodies is a highly specific but rather insensitive marker for the diagnosis of SLE (specificity almost 100%, sensitivity only 25%). Several authors have reported an association between anti-Sm antibodies and mild renal or central nervous system (CNS) involvement,[23] while others do not support any clinical or prognostic association.

Anti-Ro/SSA and anti-La/SSB antibodies belong to a group of ANAs that was originally described and detected with high frequency in sera of patients with SS. Anti-Ro/SSA antibodies were found in 30 to 40% of sera from SLE patients while anti-La/SSB were detected in much lower percentages, ranging between 10 to 15%.[11] These autoantibodies have high sensitivity and specificity for certain subsets of SLE, such as neonatal lupus erythematosus (NLE) and subacute cutaneous lupus (SCLE). In both entities, anti-Ro/SSA and anti-La/SSB antibodies have been detected in affected tissue. A strong association between maternal anti-Ro/SSA and anti-La/SSB and congenital heart block of the fetus has also been observed. It appears that these autoantibodies are transferred from the mother to the fetus through the placenta, eventually causing injury to skin and cardiac tissue.[24]

Antibodies to histones (mainly histones H1, H2B, and H4) have been reported in approximately 40% of patients with SLE and in 10 to 30% of patients with other autoimmune diseases. These autoantibodies are not specific for SLE but are detected in 95 to 100% of patients with drug-induced lupus. Thus, their absence has high diagnostic value, excluding this diagnosis with an accuracy reaching nearly 100%.[25]

Anti-ribosomal P antibodies are detected in a limited number of SLE patients (10 to 20%). Their presence appears to be associated with lupus cerebritis and psychosis.[26] Anti-phospholipid antibodies (aPL) target phospholipid-dependent factors in the coagulation cascade, such as phospholipid cardiolipin or plasma apolipoprotein β2 glycoprotein I (β2GPI) to which it binds. These antibodies are frequently detected in patients with antiphospholipid syndrome (APS). This disorder is referred to as primary APS when it occurs alone; however, it can also be found in association with SLE. The clinical hallmark of the disease is venous or arterial thrombosis. The risk of thromboembolic phenomena in APS and SLE has been previously associated with the presence of aPL antibodies, and it has also been rather closely associated with anti-β2GPI antibodies.[27]

Anti-Ku antibodies are found in a wide variety of connective tissue disorders. While some patients with these antibodies fulfilled diagnostic criteria

for SLE, scleroderma, or RA, their clinical features were usually mild and did not form a distinctive pattern. Common features associated with anti-Ku antibodies were Raynaud's phenomenon, arthralgia, skin thickening, and esophageal reflux.[28]

B. Sjogren's Syndrome

SS is a chronic autoimmune disorder characterized by lymphocyte infiltration of exocrine glands and resulting in xerostomia and keratoconjunctivitis sicca. Its clinical manifestations range from autoimmune exocrinopathy to extraglandular (systemic) involvement affecting the lungs, kidneys, blood vessels, and muscles; it may occur alone (primary SS) or in association with other autoimmune diseases (secondary SS). In approximately 5% of patients, the benign autoimmune process can tranform into a lymphoid malignancy. Thus, SS is a "cross-roads disease" that offers potential insight into the mechanisms predisposing to malignant transformation of B cells that are already involved in an autoimmune process.[29]

Extraglandular manifestations are divided in two groups: (i) lung, kidney (interstitial nephritis), and liver involvement, which are a result of lymphocytic invasion in epithelial tissues; and (ii) skin vasculitis, peripheral neuropathy, and glomerulonephritis, which are characterized by low C4 levels, the result of immune complex disease, and are associated with increased morbidity and high risk for lymphoma.[30,31] The two major autoimmune phenomena observed in SS are lymphocytic infiltration of affected tissues and production of autoantibodies. In fact, the common feature of all organs affected in SS patients is periepithelial lymphocytic infiltration, which causes functional disability and results in the various clinical manifestations. Clinical and laboratory observations point to the central role of the epithelial cell and, therefore, the etiologic name of the disease has been proposed to be "autoimmune epithelitis."[32]

Clinical diagnosis relies on a combination of subjective symptoms, objective tests of secretory function, salivary gland histology, and evidence of autoantibody production. The prevalence of primary SS in the general population is around 0.6%.[33] Intense research on immunopathology, autoantibodies, and immunogenetics has further refined the concepts of the pathophysiology and pathogenesis of primary SS.[29] Recent work from our laboratory has reported enteroviral sequences in the epithelial cells of primary SS patients but not in those of controls.[34]

The gold standard for diagnosis is a minor salivary gland biopsy that demonstrates focal lymphocytic infiltrates. Most criteria require more than one infiltrate focus per 4 mm^2 for a definitive diagnosis of SS.[6] The infiltrates are dominated by CD4 T cells releasing Th-1 cytokines. Activated B cells producing autoantibodies have been also found.[29,35]

TABLE 2 Common Autoantibodies in Sjogren's Syndrome

Disease	Autoantibody target	Frequency (%)
Sjogren's syndrome	ANA	55
	Ro/SSA*	70
	La/SSB*	60
	RF	85
	a-fodrin	30

*Included in the European-American criteria for the diagnosis of SS.

The most common serological finding in SS is hypergammaglobulinemia. Sera of patients contain a number of autoantibodies directed against non-organ-specific antigens (Table 2), such as other immunoglobulins (rheumatoid factor, RF), ANA, which usually give a speckled pattern on immunofluorescence, and cellular antigens [Ro/SSA, La/SSB, RANA] as well as organ-specific antigens, such as salivary ductal cell, thyroid gland cell, and gastric mucosal antigens. Recently, new autoantibodies directed against the cytoskeletal proteins α and β-fodrin; the proteasomes; the muscarinic receptors M3; and a newly-identified protein, SS-56, which is structurally related to the 52-kDa Ro/SSA antigen, have been described in primary SS. The most common autoantibodies to cellular antigens in patients with SS are directed against two RNP antigens known as Ro or SSA and La or SSB. These autoantibodies are not specific for the syndrome and may be found in other autoimmune diseases, especially SLE. Anti-Ro and anti-La are detected in 45 to 70% and 20 to 50%, respectively, of patients with SS. The presence of anti-Ro/SSA and La/SSB autoantibodies is associated with earlier onset and longer duration of disease, parotid gland enlargement, splenomegaly, lymphadenopathy, and vasculitis. The incidence of these antibodies also correlates with the intensity of the infiltration of minor salivary glands.[36,37]

C. Rheumatoid Arthritis

RA is a chronic, systemic, inflammatory disorder that involves primarily joints. Its typical clinical presentation is symmetrical arthritis that may be remitting but, if uncontrolled, may lead to deformity and destruction of joints due to erosion of cartilage and bone. It is the most common form of inflammatory arthritis in adults, affecting about 1% of Caucasians, with a female-to-male ratio of 3:1. In the majority of patients, RA results in significant locomotor disability within 10 to 20 years. The pathogenesis of RA is still incompletely understood. The pathology of the disease includes chronic synovial inflammation and pannus formation, consisting of macrophages, fibroblasts, T and B lymphocytes, synoviocytes, and mast cells. Direct tissue invasion by pannus is thought to be responsible for the ongoing cartilage and bone destruction and joint deformity.

Early diagnosis is important in RA, since delay in appropriate therapeutic intervention can lead to rapid joint damage and a less favorable outcome, whereas early treatment appears to improve the outcome.[39] The diagnosis is dependent upon the demonstration of four or more of the ACR-revised diagnostic criteria for the disease. Only one out of seven criteria for RA refers to laboratory assessment, which is the presence of circulating RF, autoantibodies that target to the Fc portion of IgG.[40] They are found in 75 to 80% of RA patients at some time during the course of their disease. RFs are also present in sera of patients with other autoimmune diseases such as SS, SLE, mixed cryoglobulinemia, and mixed connective tissue disease (MCTD) as well as in non-rheumatologic conditions including infective endocarditis, lymphoproliferative disorders, and old age. RF may be a prognostic factor in established RA because a high titer of RF has been associated with more severe and extra-articular disease, such as vasculitis.

Another common autoantigen specificity in RA is fillagrin, a cytokeratin filament aggregating protein of the epidermis. This protein is the common antigenic target for anti-perinuclear factor (APF) and anti-keratin antibodies (AKA), which were considered for many years to be different autoantibody specificities in sera of RA patients.[41,42] Anti-fillagrin autoantibodies are detected in approximately 70% of patients and mainly target post-translationally modified structures in fillagrin that contain citrulline.[43,44] Antibodies to cyclic citrullinated peptides are detected in RA patients' sera long before the onset of disease and are associated with erosive disease.[39] BiP, a protein that serves as a chaperone in the endoplasmic reticulum and binds to immunoglobulin heavy chains, has recently been identified as a common autoantibody target in RA. However, the diagnostic value of these autoantibodies is not well established.[45] The most common autoantibody specificities in RA are summarized in Table 3.

D. Systemic Sclerosis (SSc)

Systemic sclerosis (scleroderma, SSc) encompasses a spectrum of related disorders of unknown etiology. The simplest classification of the scleroderma-related disorders is into localized (*e.g.*, morphea, linear scleroderma) and

TABLE 3 Common Serologic Findings in Rheumatoid Arthritis

Disease	Autoantibody target	Frequency (%)
Rheumatoid arthritis	ANA	25–35
	RF	75–80*
	Fillagrin	70
	Anti-Bip(p68)	65
	Ro/SSA	5–14

*Included in the ACR criteria for the diagnosis of RA.

systemic (*e.g.*, limited cutaneous SSc, lSSc, with no truncal involvement or diffuse cutaneous SSc, dSSc) forms of the disease.[46] The CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and teleangiectasia) is a subset of SSc. The prevalence of SSc was estimated to be 10 to 20 cases per 100,000 population. It strikes predominantly adults, with a female-to-male ratio of 3:1. The clinical expression of the disease is heterogeneous and ranges from mild disease to a devastating ischemic and fibrotic process leading to severe organ damage, predominantly in the lungs, heart, or kidneys. The pathological hallmark of scleroderma is vascular damage, accumulation of mononuclear leukocytes around blood vessels, and fibrosis in tissues and visceral organs.

ANA are detected in over 95% of patients with established SSc. ANA testing in patients suspected of having SSc is recommended because a negative result prompts consideration of other fibrotic disease.[47] If ANA is positive, then a more elaborate search for the responsible autoantigen is useful, since approximately 75% of patients with scleroderma have circulating autoantibodies directed against a number of autoantigens. These include topoisomerase I (formerly called Scl-70), centromere antigens (25 to 30%), and RNA polymerase I, II, and III (20%). Although not very sensitive, anti-topoisomerase I antibodies are highly associated with diffuse scleroderma and pulmonary fibrosis.[48] Anti-centromere antibodies (ACA) are associated with CREST syndrome. The sensitivity of ACA is 32 and 57% for the diagnosis of scleroderma and CREST syndrome, respectively. However, ACA are detected in patients with SLE as well as in some patients with idiopathic Raynaud's phenomenon. Anti-RNA polymerase I and III are found only in patients with SSc whereas antibodies to RNA polymerase II are found in patients with either SSc or SLE.[49] The common serologic findings in SSc are summarized in Table 4.

E. Mixed Connective Tissue Disease

MCTD was originally described as a connective tissue disorder characterized by the presence of high titers of a distinctive autoantibody, now called anti-U1 small nuclear ribonuclear protein particles (snRNP).[50] MCTD

TABLE 4 Autoantibodies in Systemic Sclerosis

Disease	Autoantibody target	Frequency (%)
Diffuse cutaneous systemic sclerosis	Scl-70	20–30
	RNA polymerase I, II, III	20
	U1 snRNP	10
Limited cutaneous systemic sclerosis CREST	Centromere	70–90

TABLE 5 Autoantibodies in Mixed Connective Tissue Disease

Disease	Autoantibody target	Frequency (%)
Mixed connective tissue disease	ANA	>95
	U1 snRNP*	>90
	dsDNA	10
	Ro/SSA	10

*Prerequisite for the diagnosis of MCTD.

is considered an overlap syndrome, with clinical characteristics resembling those of SLE, RA, polymyositis/dermatomyositis (PM/DM), and SSc. Common early features are Raynaud's phenomenon, puffy hands, arthritis in the small joints of the hands, and serositis. During the disease course, evolution to one of the aforementioned systemic rheumatic diseases can occur.[51] The first clue to the diagnosis of MCTD is the presence of Raynaud's phenomenon, puffy hands, and arthralgias in a patient who is positive for ANA at a high titer; the ANA gives a speckled pattern on immunofluorescence, due to anti-U1 snRNP antibodies. Detection of these antibodies is a prerequisite for diagnosing MCTD, although they may also be present in SLE (prevalence 30 to 40%), RA, PM, or scleroderma.[52] Long-term follow-up of patients with MCTD has demonstrated that many of these patients eventually develop SLE, RA, scleroderma, or other rheumatic disease, indicating that anti-U1 snRNP antibodies may be less specific for MCTD than was initially thought. These observations prompt some investigators to consider MCTD to be part of SLE and not a distinct entity. The common serologic findings in MCTD are summarized in Table 5.

F. Idiopathic Inflammatory Myopathies

Idiopathic inflammatory myopathies consist of DM, PM, and inclusion body myositis (IBM).[53] DM and PM are rare disorders with prevalence rates estimated at about one per 100,000 in the general population. There is also a female-to-male ratio of about 2:1.[54]

Idiopathic inflammatory myopathies comprise the best group of diseases classified according to autoantibody specificities. In fact, PM/DM-specific autoantibodies are useful in defining clinically homogenous subsets of patients.[55] The most prevalent myositis-specific antibody, the anti-histidyl-transfer RNA (tRNA) synthetase (anti-Jo-1 antibody), is one of a group of anti-tRNA synthetase autoantibodies (Table 6). It is present in 25 to 30% of cases and is strongly associated with interstitial lung disease, Raynaud's phenomenon, arthritis, and mechanic's hands, a syndrome also called "antisynthetase syndrome". Patients with antibodies to the nuclear antigen Mi-2 (a helicase involved in transcriptional activation) have a less acute onset of disease, often display the classical rash of DM, and generally have a good

TABLE 6 Common Serologic Findings in Inflammatory Muscle Diseases

Disease	Autoantibody target	Frequency (%)
Dermatomyositis/ polyomyosistis	ANA	80
	Jo-1 (Histidyl-tRNA synthetase)	25–30
	PL-7 (Threonyl-tRNA synthetase)	1–2
	PL-12 (Alanyl-tRNA synthetase)	1
	EJ (Glycyl-tRNA synthetase)	1
	OJ (Isoleucyl-tRNA synthetase)	1
	KS (Asparaginyl-tRNA synthetase)	<1
	Mi-2	7

response to therapy. Antibodies to signal recognition pattern (anti-SRP) target an RNP involved in translocation. Patients with this autoantibody have progressive PM that is refractory to treatment as well as increased frequency of cardiac involvement.[56] Anti-PM/Scl is an anti-nucleolar antibody that identifies a subset of patients with myositis who also have features of SSc.

G. Systemic Idiopathic Vasculitis

Systemic vasculitides comprise a group of diseases characterized by inflammatory cell infiltration and necrosis of the blood vessel wall. The severity of vasculitis is related to the size, site, and number of vessels affected. In general, affected vessels vary in size, type, and location in association with the specific vasculitic disorder. Vasculitic syndromes are classified according to the size of the affected blood vessel as follows: (a) large vessel vasculitis: Takayasu arteritis, giant-cell arteritis; (b) medium vessel vasculitis: polyarteritis nodosa, Kawasaki disease; and (c) small vessel vasculitis: Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyarteritis, Henoch-Schonlein purpura, cryoglobulinemic vasculitis, vasculitis secondary to connective tissue disorders, vasculitis secondary to viral infection.[57] The clinical presentation of primary vasculitis depends largely on the type and size of the vessel involved, although complaints including fatigue, weakness, fever, arthralgias, abdominal pain, hypertension, and renal insufficiency are commonly present.

Specific types of small vessel vasculitis (namely Wegener's granulomatosis, Churg-Strauss syndrome, and microscopic polyarteritis) are associated with anti-neutrophilic cytoplasmic antibodies (ANCA), which are directed against neutrophil cytoplasmic antigens.[58] The annual incidence of ANCA-associated vasculitides is 10 to 30 per 1,000,000 people. The vasculitides are characterized by necrotizing systemic vasculitis and the presence of ANCA.[59] The two target antigens of ANCA antibodies are proteinase 3 (PR3) and myeloperoxidase (MPO). Both PR3 and MPO are located in the azurophilic granules of neutrophils and the peroxidase-positive lysosomes of monocytes. Antibodies with target specificities for PR3 and MPO produce c-ANCA

TABLE 7 Prevalence of ANCA in Systemic Vasculitis

Disease	ANCA positive (%)	Immunofluorescence pattern	Major antigen
Wegener's granulomatosis	90	c-ANCA	PR3
Microscopic polyarteritis	70	p-ANCA	MPO
Churg–Strauss syndrome	50	p-ANCA	MPO

and p-ANCA immunofluorescence patterns respectively in freshly fixed neutrophils (see below). In Wegener's granulomatosis, ANCA are primarily directed against PR3, whereas in Churg-Strauss syndrome and microscopic polyarteritis, ANCA are in most cases directed against MPO. Determination of ANCA can also be useful for monitoring disease activity because disease relapses are often preceded by rises in ANCA levels.[60] Furthermore, persistingly high levels of ANCA are associated with severe renal damage.[61] The common serologic findings in ANCA-associated vasculitides are summarized in Table 7.

III. CLASSIFICATION AND CHARACTERISTICS OF B-CELL EPITOPES OF AUTOANTIGENS

A. The Value of B-Cell Epitopes

During the past fifteen years, many laboratories have attempted to define the B-cell epitopes of intracellular autoantigens. The purpose of this endeavor[62,63] was to:

1. Understand in detail the antigenic structures targeted by autoantibodies, in order to provide information on the mechanisms involved in the breakdown of immune tolerance and the establishment of the autoimmune response. Detailed analysis of the epitopes may also disclose homologous sequences shared among autoantigens and foreign proteins that may cross-react with the same antibody, producing the phenomenon of molecular mimicry.
2. Study the mechanisms of epitope spreading in both human sera and experimental animals in order to achieve further insights into the understanding of the perpetuation and augmentation of the autoimmune response.
3. Develop sensitive and specific methods for autoantibody detection. The use of synthetic peptides can offer an alternative source of antigen of high purity and stability at low cost. Furthemore, synthetic peptides can be easily engineered to fit in various detection assays since they can be

readily modified by the attachment of a biotin, aminocaproic acid, or carboxy-terminal cysteine moiety in order to bind to streptavidin, hydrophobic, or maleimide enzyme-linked immunosorbent assay (ELISA) plates, respectively.

4. Study the association of autoantibodies against a specific epitope with the clinical picture or certain clinical findings of a given autoimmune disease.
5. Develop potential therapeutic applications, including the generation of peptide-based vaccines and immunoabsorption columns for the removal of specific pathogenic autoantibodies as well as the use of complementary (or antisense) peptides to induce anti-idiotypic response.

B. Types of B-Cell Epitopes

The B-cell epitopes are classified according to their structure as follows:

1. *Primary-structure epitopes (identified also as linear epitopes)* are composed of sequential amino acids. Such epitopes have been identified by synthetic peptide mapping in the majority of autoantigens discussed here, including Ro60, Ro52, La, Sm B, Sm D, RNP70, and Scl-70.
2. *Secondary-structure epitopes* are formed by amino acids distributed in simple three-dimensional structures, such as α-helices or β-sheets. These epitopes have been identified in the PM/Scl-100 autoantigen. They reside in a local α-helical secondary structure stretch with all amino acids relevant for antibody binding located on one side of the helix. Other secondary-structure epitopes have been described in Ro52 and Ro60 leucine zipper and zinc finger motifs, in patients with neonatal lupus and primary SS, respectively.
3. *Tertiary-structure epitopes* are formed by distant regions of the protein sequence coming together in the tertiary structure. Such conformational epitopes may be the main targets of some autoantibodies (*e.g.*, anti-Ro60).
4. *Quaternary-structure epitopes* consist of amino acids distributed over different subunits within a macromolecular complex, interacting transiently or permanently to form a structure recognized by the autoantibody. Such epitopes have been identified in the Ro/La RNP complexes as well as in nucleosome subunits composed of histones and DNA elements.
5. *Cryptic epitopes (cryptotopes)* are usually linear epitopes hidden in the native structure of the autoantigen. They become accessible to antibody binding after disruption of the three-dimensional structure by denaturation, proteolytic degradation, or chemical modification of the autoantigen. These epitopes are observed in a number of nuclear autoantigens.
6. *Modified epitopes (neoepitopes)* contain post-translationally modified amino acid residues.[64] Examples of these modifications include: (i) serine, threonine and tyrosine phosphorylation; (ii) lysine acetylation

or ubiquitination; (iii) cysteine lipidation or oxidation (disulphide-bond formation); (iv) glutamic acid methylation or γ-carboxylation; (v) glutamine deamidation; (vi) asparagine (N-linked) and serine/threonine (O-linked) glycosylation; (vii) arginine citrullination or symmetric/asymmetric dimethylation; and (viii) proteolytic cleavage or degradation. In some instances, side chain modifications of specific amino acids, such as citrullination of arginine residues, are responsible for epitope high-affinity binding.[62] Such modified amino acids have been reported in a variety of human nuclear proteins, including the Sm antigens D1 and D3,[65] fibrillarin,[66] and nucleolin.[67]

IV. B-CELL EPITOPES OF INTRACELLLAR AUTOANTIGENS: BIOLOGIC PROPERTIES AND CLINICAL ASSOCIATIONS

A. Ro/La RNP Particles

In patients with SLE and SS, one of the main targets of autoantibodies is the Ro/La RNP (Figure 1). Human Ro/La RNP is composed of one of the four small, uridine-rich hY RNAs (**h**uman c**Y**toplasmic **RNA**) noncovalently associated with at least three proteins, the Ro52, La, and Ro60 autoantigens.[68,69] Additional components of the complex have been identified recently as the proteins calreticulin[70] and nucleolin.[71]

The localization of these complexes is exclusively cytoplasmic but their protein components can be found in the nucleus as well. In the nucleus, the Ro60, Ro52, and La autoantigens are not associated with hY RNA. After

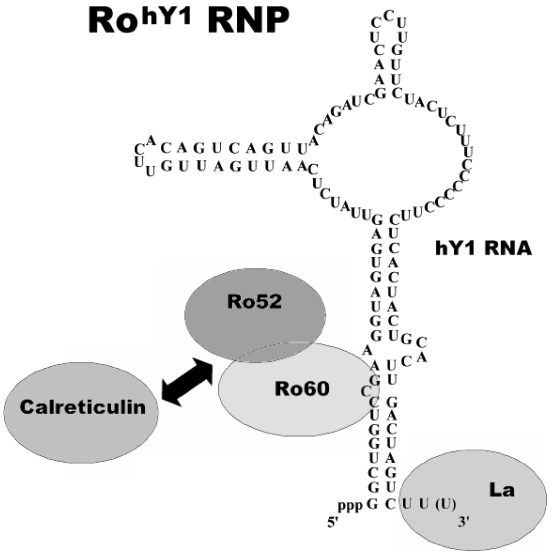


FIGURE 1 Structure of Ro^{hY1} ribonucleoprotein particle.

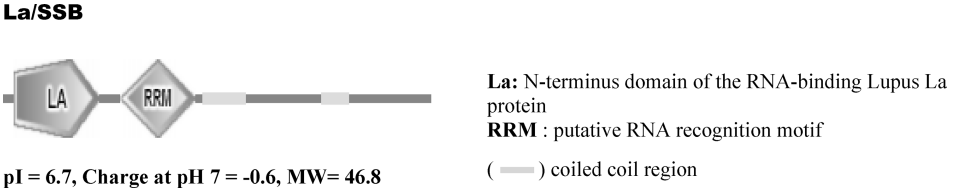


FIGURE 2 Domain organization of the autoantigen La.

the assembly of the Ro RNP in the nucleus, the complex is rapidly and quantitatively transported to the cytoplasm.[72,73]

B. The La Autoantigen

The La/SSB antigen (Figure 2) is a 47-kDa phosphoprotein that associates with a variety of small RNAs, including 5S cellular RNA and tRNA, 7S RNA, and hY RNAs. The protein binds to the 3′-terminal end of the RNAs and acts as a transcription termination factor for RNA polymerase III.[72–74] Thus, La mediates transcript release from RNA polymerase III and facilitates multiple rounds of transcription reinitiation by RNA polymerase III. In addition, immature RNA polymerase III products and hY RNAs La bind viral RNAs (*e.g.*, adenovirus VA, Epstein Barr EBER), viral and human RNAs possessing internal ribosomal entry site (IRES), and the RNA component of telomerase complex.[75,76] La/SSB autoantigen has other cellular functions: (i) La is a factor for cap-independent translation (La binds to the 5'-untranslated region of viral or human mRNAs possessing IRES elements and promotes their internal, cap-independent initiation of translation at the correct AUG);[77] (ii) La acts as an adenosine triphosphate (ATP) dependent helicase able to melt RNA-DNA hybrids;[78] (iii) La unwinds double-stranded RNA inhibiting the double-stranded RNA-dependent activation of protein kinase PKP;[79] (iv) La can associate with telomerase and influence telomere homeostasis *in vivo*;[76] and (v) La is an RNA chaperone capable of transient bipartite (5′- and 3′- end) binding of nascent transcripts synthesized by polymerase III (*e.g.*, tRNA precursors).[80] Phosphorylation of serine-366 has been shown to play an essential role in La functions (*e.g.*, La phosphorylated at this position appears to be transcriptionally inactive whereas dephosphorylated La is active).

During the last decade, B-cell epitopes of La/SSB have been mapped by several investigators. Early efforts to identify epitopes on the La antigen began by using enzymatic digestion of the native protein. In this instance, antigenic sites covering the larger part of La autoantigen were identified. These sites were called LaA (amino acids 1–107, 1–107aa), LaC (111–242aa), and LaL2/3 (246–408aa).[81] Later, more detailed and analytical epitope mapping revealed the exact localization of its antigenic determinants. Some of the La epitopes were found to reside in functional regions of the autoantigen, like the RNA recognition motif (RRM) and the ATP binding site.[82–84] The

interaction of hYRNA with the RRM motif, however, did not affect autoantibody binding in the same region.[82] In contrast, the interaction of the ATP binding site with ATP abolishes autoantibody binding at the same part of the protein.[84] B-cell epitope mapping of La/SSB was also performed in our laboratory using 20-mer synthetic peptides overlapping by eight amino acids and covering the whole sequence of the protein. Peptides highly antigenic were those spanning the sequences; HKAFKGSI(147–154aa) (located within the RRM motif: 113–182aa), NGNLQLRNKEVT (291–302aa), VTWEVLEGEVEKEA LKKI(301–318aa) and GSGKGKVQFQGKKTKF (349–364aa).[85] The most sensitive and specific epitope, 349–364aa (with >90% sensitivity and specificity), was synthesized attached to a tetramer sequential oligopeptide carrier SOC_4 and was used for immunoassay development. Ninety % of anti-La positive sera were reactive with both the synthetic peptide, 349–364aa, and the recombinant La protein.[86] Thus, this epitope analogue exhibited comparable utility with the recombinant La/SSB for the detection of anti-La/SSB antibodies. It was also found that autoantibodies to the La/SSB epitope, 349–364aa, were positively-associated with longer disease duration, recurrent or permanent parotid gland enlargement, and a higher proportion of non-exocrine manifestations compared to SS patients without autoantibodies.[87]

C. The Ro60 Autoantigen

Ro60 antigen (Figure 3) is proposed to function in the quality control or discard pathway for nascent transcripts synthesized by RNA polymerase III (*e.g.*, 5S rRNA precursors). Epitopes of Ro60 have been described by several authors using a variety of epitope mapping procedures.[9,88] Three major studies[89–91] using recombinant fragments of Ro60 identified a major epitope within the central part of the molecule (within 181–320aa, 139–326aa, and 155–295aa regions of the sequence, respectively). The exact locations of the antigenic determinants were revealed only after the application of synthetic peptides. Wahren-Herlenius *et al.*[88] identified a major epitope using synthetic peptide 216–245aa. Scofield and associates, using synthetic octapeptides, identified numerous epitopes covering the entire length of Ro60;[92,93] it appeared, in the sera used in this study, that epitope spreading to the entire Ro60 antigen had already occurred. The same investigators reported

TROVE domain: This presumed domain is found in TEP1 and Ro60 proteins that are RNA-binding components of Telomerase, Ro and Vault RNPs. This domain has been named TROVE, (after Telomerase, Ro and Vault). This domain is probably RNA-binding.

FIGURE 3 Domain organization of the autoantigen Ro60.

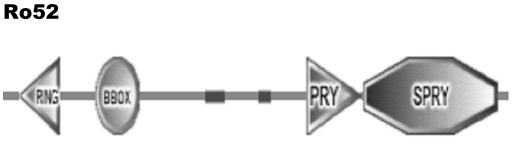
that one of the peptides (485–492aa) shared sequence similarity with the N-protein of vesicular stomatitis virus (VSV) and speculated that VSV might be involved in the pathogenesis of SLE.[90] However, in a subsequent study, the population of anti-Ro60 antibodies directed against the above-mentioned region was found to be rather limited.[94]

In our laboratory, epitope mapping with synthetic peptides revealed the precise antigenic regions of Ro60, in the 169–190aa and 211–232aa parts of the antigen.[95] One of them, the 169–190aa epitope (originally described as SLE-associated epitope), was found to share conformational and antigenic similarity with the HLADR3 β-chain, an HLA class II allele, which was described to be highly associated with the anti-Ro60 response.[96] The same epitope was recently found to be the initial pre-disease target of autoantibodies in individuals who developed SLE several years later. Our recent results indicate that although the 169–190aa and 211–232aa epitopes were identified as small peptide moieties (22 aa in length), their recognition by autoantibodies is conformation-dependent, and their antigenicity is dramatically enhanced upon interaction with the molecular chaperone, calreticulin.[97]

Recent studies in our laboratory have also focused on the zinc finger motif of the Ro60 protein. Synthetic peptide analogues corresponding to the zinc finger motif of Ro60, spanning the region 301–327aa, were prepared and tested. We found that the majority of anti-Ro/SSA and La/SSB positive sera from patients with primary SS reacted with the full-length peptide in the absence of zinc ions. In contrast, the native form of the zinc finger domain, in the presence of zinc ions, could bind to Ro52, but not to autoantibodies.[98]

D. The Ro52 Autoantigen

Ro52 functions as a transcription modulator due to its domain organization (Figure 4).[99] The two zinc-finger motifs in Ro52 are not of the types



pI = 5.9, charge at pH 7 = -8.7, MW= 54.2
RING: The RING-finger is a specialised type of Zn-finger that binds two atoms of zinc, and is probably involved in mediating protein-protein interactions.
BBOX: The B-box zinc finger is a motif generally associated with a ring finger and a coiled coil motif to form the so-called tripartite motif. It is found essentially in transcription factors, ribonucleoproteins and protooncoproteins.
SPRY: The SPRY domain is named from SPla and the RYanodine Receptor and its function is unknown.
PRY: PRY is a domain of unknown function associated with SPRY domains.

(▬) segment of low compositional complexity
The middle part of Ro52 comprises a coiled-coil domain. Within this domain a leucine zipper motif is situated (aa 211-232).

FIGURE 4 Domain organization of the autoantigen Ro52.

that promote binding of nucleic acid but instead usually promote protein-protein-interactions.[100] DNA-binding activity has, however, been suggested for Ro52, and a consensus binding motif has been described. In line with many other RING-containing proteins, a role for Ro52 in ubiquitination has been suggested.[101] Ro52 can also homodimerize through its leucine zipper domain.[102]

The epitopes of Ro52 have been mapped in various studies with different methods. The major immunoreactivity of Ro52 autoantigen was localized, using recombinant Ro52 fusion proteins, in the middle coiled-coil region of Ro52.[103–105] The 190–245aa region of the sequence was reactive with almost all anti-Ro52 positive sera and was independent of associated diseases.[103] An epitope spanning the 200–239aa region of Ro52, which contains the complete leucine zipper, has also been identified.[106] Autoantibodies against this epitope were associated with NLE and congenital heart block. These autoantibodies can bind directly to the cell surfaces of cardiomyocytes in primary culture and cause dysregulation of the Ca2+-homeostasis, which is followed by apoptosis.[107]

E. The Multifunctional Chaperone Calreticulin

The protein calreticulin (Figure 5) is an exemplary multifunctional molecule of the endoplasmic reticulum capable of interacting with proteins, peptides, sugars, and nucleic acids.[108] The exact mode of interaction with other Ro/La RNP complex components is controversial since it has been found to interact with Ro52, hY RNA, and the epitopes of Ro60 autoantigen.[70,97] Some of the numerous functions of calreticulin include: (i) regulation of Ca^{2+} homeostasis; (ii) chaperoning of glycoproteins in the endoplasmic reticulum to ensure proper folding; (iii) action as a stress protein; (iv) regulation of integrin-mediated adhesion (surface calreticulin); (v) modification of gene expression by binding to the glucocorticoid receptor; (vi) a

Calreticulin: is a calcium-binding protein organized in three domains: an N-terminal, probably globular, domain (N-domain) of about 180 amino acid residues; a central domain (P-domain) of about 70 residues which contains three repeats of an acidic 17 amino acid motif, and which binds calcium with a low-capacity, but a high-affinity; a C-terminal domain (C-domain) rich in acidic residues and in lysine, which binds calcium with high-capacity but low-affinity.

(——) coiled coil region

(▬)Signal peptide

FIGURE 5 Domain organization of the autoantigen calreticulin.

role as a component of cytotoxic T-lymphocyte/natural killer cell (CTL/NK) granules; and (vii) action as C1q receptor (surface calreticulin).[108]

Its immunoreactivity, which is rather limited, involves antigenic regions in the N-terminous (1–24aa) and the central part of the molecule (193–207aa, 253–282aa).[109,110] However, if Ro epitopes are complexed together with calreticulin, the antigenicity of the complex is increased compared to that of calreticulin or the Ro epitopes alone.[97] Using complexes of highly-purified human calreticulin with the linear epitopes of Ro60, almost all positive anti-Ro60 sera were found to bind strongly onto the newly-formed conformation of the epitopes.[97] When calreticulin or the linear epitopes of Ro60 were tested individually with the same sera, the prevalence of positive reactions was much lower. These observations suggest conformation-dependent enhancement of antigenicity of the Ro60 epitopes upon interaction with the chaperone protein calreticulin. Such complexes can potentially be used as substrates for the efficient detection of autoantibodies.

F. The U1 snRNP Particle

The U1 snRNP particle (Figure 6) is a major component of the spliceosome, the complex macromolecular machinery that catalyzes pre-mRNA splicing into messenger RNA. Its 3-dimensional architecture was recently defined with cryo-electron microscopy.[111] Together with the Sm proteins, it contains three specific proteins, denoted RNP 70, RNP A, and RNP C, that

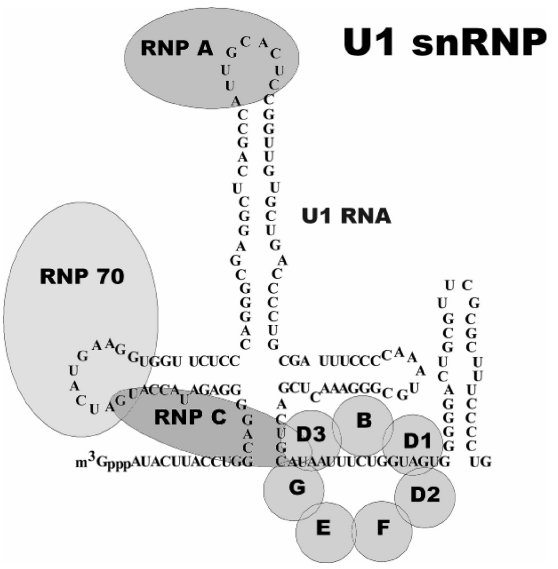


FIGURE 6 Structure of U1 snRNP ribonucleoprotein particle.

associate with the 164-nucleotide long U1 RNA. The RNP 70 and RNP A proteins, which contain classical RRMs, bind directly to the U1 RNA, whereas the RNP C protein associates via protein-protein interactions with RNP 70 and one or more of the Sm proteins.[75] The 7 Sm core proteins, B, D1, D2, D3, E, F, and G, form a heptamer ring approximately 20 nm in diameter, with the U1 RNA passing through the center.[112] Remarkably, all the antigenic protein components of the U1 snRNP complex are positively-charged (pI: 9.4–12.1) with the exception of Sm F, which is negatively charged (pI: 4.4).

G. The RNP 70, A and C Autoantigens

The antigenic properties of RNP 70 A and C polypeptides (Figure 7) have been studied by several investigators. Similar to the 147–154aa epitope of La/SSB, described above, the major B-cell epitopes of RNP 70 and RNP A autoantigens are located within their RNA binding domain (RRM).[113–119] In RNP 70, these epitopes are multiple and most probably discontinuous. However, a linear epitope resides in 119–126aa of this region. Additionally, a new conformational epitope can be generated in the RRM of RNP 70 after it binds to U1 RNA.[118] This quaternary-structure epitope has been found to be reactive with sera from patients with MCTD. In RNP A autoantigen (Figure 8), the epitope in its RRM motif (35–58aa region) possesses characteristics of

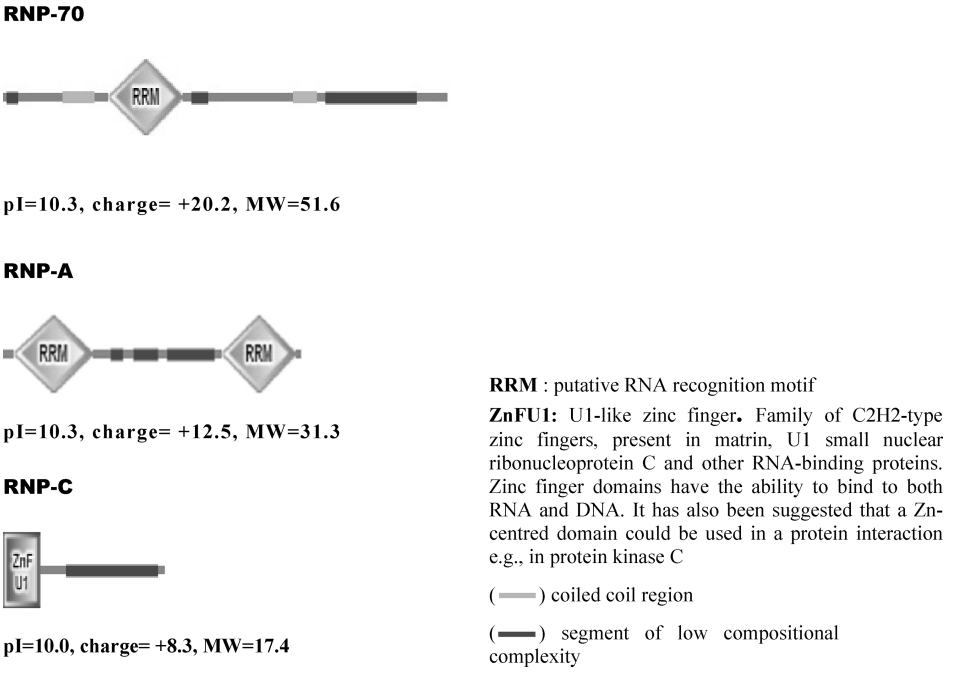


FIGURE 7 Domain organization of the autoantigens RNP 70, A, and C

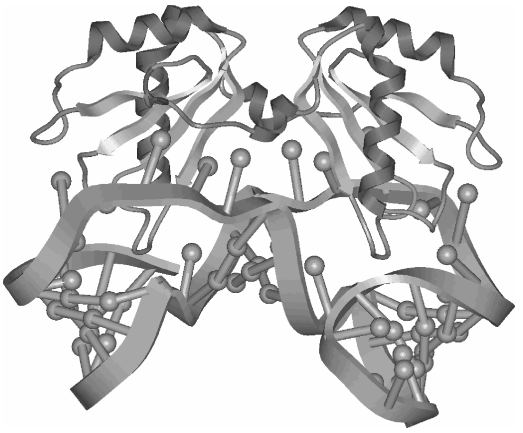


FIGURE 8 Model of the RNP A (2–97aa) complexed with pre-mRNA (PDB: 3UTR) (PDB is protein database, alpha-numeric code is protein code: see Expert Protein Analysis System proteomics server, Swiss Institute of Bioinformatics (SIB) at http://kr.expasy.org).

a disease-specific epitope, reacting with the sera of 94% of MCTD and only 19% of SLE patients.[119] Another epitope located in its proline-rich sequence (166–172aa, PPGMIPP) is highly homologous with epitopes on RNP C and Sm autoantigens.[120]

U1 RNP C lacks an RRM motif and does not bind to U1 RNA, but it is indirectly associated with it via its interaction with RNP 70 and one or more of the Sm proteins. The major epitope of the U1 snRNP C antigen is located in its 102–125aa region, which contains the proline-rich sequence APGMRPP (119–125aa).[121–123] This epitope is highly homologous with proline-rich epitopes on RNP A, Sm B, Sm B', and Sm N autoantigens and can be cross-recognized by autoantibodies targeting them.[124]

H. The Sm Autoantigens

In Sm B/B' autoantigens (Figures 9, 10), the major epitope resides in the proline-rich sequence PPGMRPP repeated 3 times in its carboxy-terminal region. Antibodies against this epitope can cross-recognize different components of the U1 snRNP particle and can potentially be detected by a single peptide.[125] A sensitive, highly-reproducible ELISA was developed in our laboratory to investigate whether the synthetic heptapeptide PPGMRPP, anchored in five copies to a sequential oligopeptide carrier (SOC), [$(PPGMRPP)_5$-SOC_5] is a suitable antigenic substrate to identify this subgroup of autoantibodies.[126] The sensitivity and specificity of the method were 98% and 68%, respectively, for the determination of anti-Sm antibodies, while for the determination of anti-Sm and/or anti-U1 RNP reactivity (antibodies to snRNPs), the corresponding values were 82% and 86%, respectively.

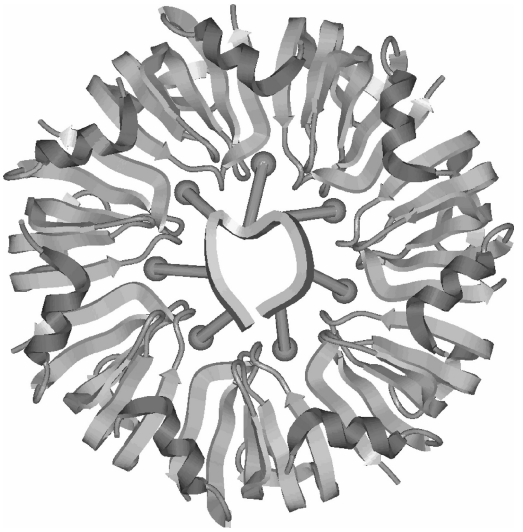


FIGURE 9 The heptamer ring of Sm core proteins in complex with a uridine RNA heptamer (PDB: 1M8V).

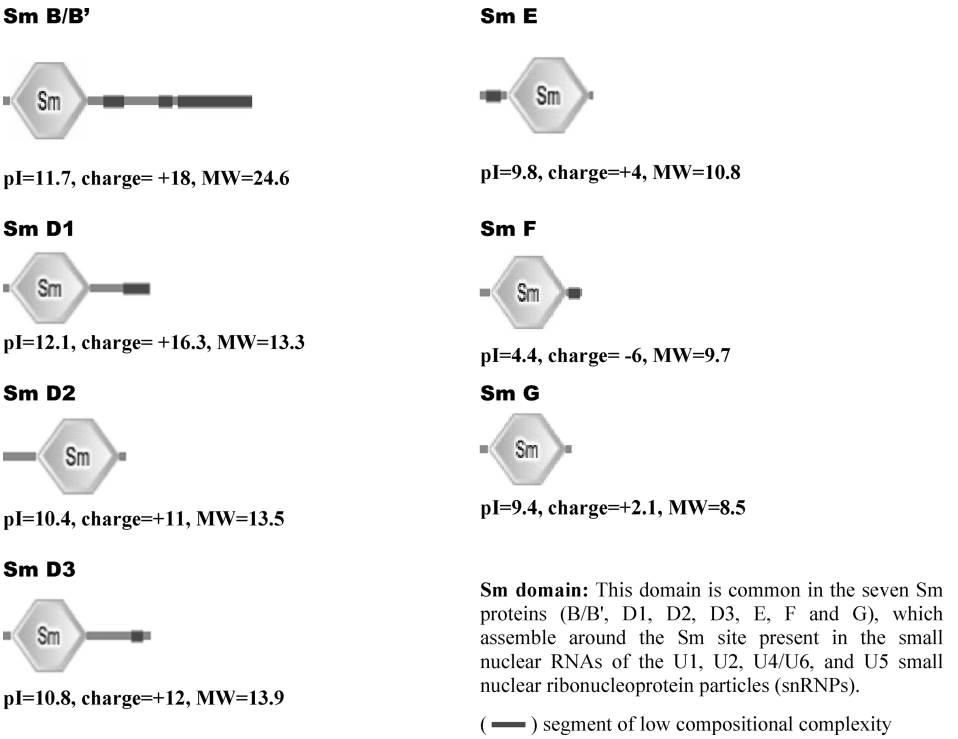


FIGURE 10 Domain organization of the autoantigens Sm B/B', D1, D2, D3, E, F, and G.

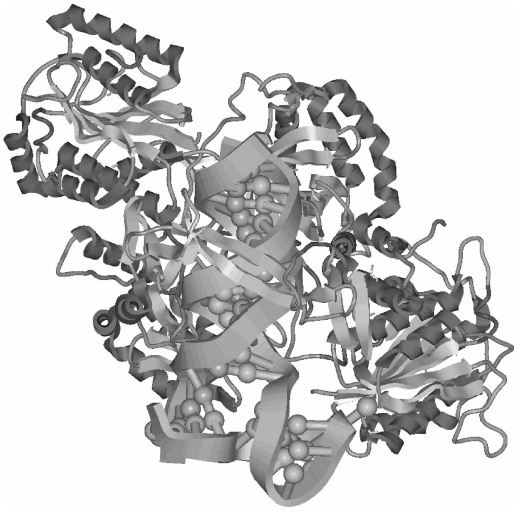


FIGURE 11 Structure of the Ku heterodimer bound to dsDNA (PDB: 1JEY).

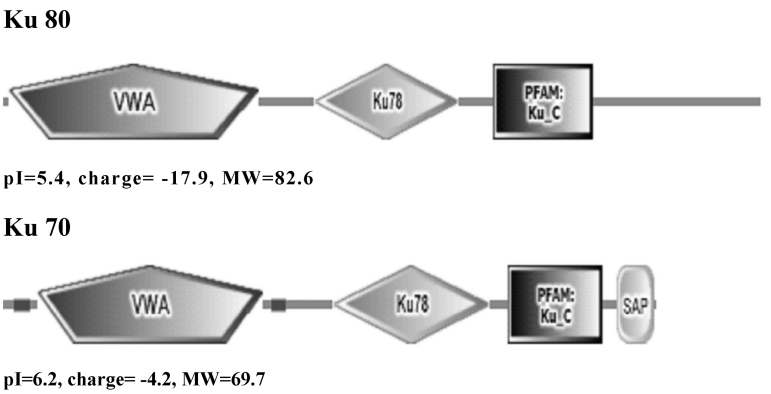
Other Sm epitopes, primarily on the C terminus of the B, D1, and D3 proteins have also been identified. Recently, dimethylarginine-modified CRG repeats on the B, D1, and D3 autoantigens have been identified as major epitopes.[63] These post-translationally modified sequences have been proposed as another target of cross-reacting autoantibodies in the U1 snRNP particle.

I. The Ku Autoantigen

Ku is found throughout eukaryotic evolution, and in higher eukaryotes is associated with the DNA-dependent PK catalytic subunit to form the DNA-PK holoenzyme. DNA-PK phosphorylates chromatin-bound proteins and is involved in dsDNA break repair, VDJ recombination, and isotype switching. Ku is a heterodimer composed of two subunits—Ku70 and Ku80—that bind to DNA ends in a sequence-independent manner (Figure 11).[127] Assembly of the Ku heterodimer is required to obtain DNA binding activity and association with DNA-PK. Heterodimerization of the Ku antigen involves the 1–115aa and 430–482aa regions of the Ku70 and the central part (371–510aa) of the Ku80 autoantigen. The immunoreactivity of the Ku70 and Ku80 autoantigens is localized mainly in their extreme carboxyl-terminal region (Figure 12).[127–132] In the case of Ku70, the major epitope also resides within the DNA binding (SAP) domain of the autoantigen.[131]

J. Ribosomal P Proteins

Among ribosomal constituents, the acidic ribosomal P proteins (Figure 13) are the only molecules that exist in multiple copies. P proteins



VWA: von Willebrand factor type A domain. VWA domains in extracellular eukaryotic proteins mediate adhesion via metal ion-dependent adhesion sites (MIDAS). Intracellular VWA domains and homologues in prokaryotes have recently been identified.
Ku78: This domain exist in 70kDa and 80kDa subunits of the Lupus Ku autoantigen This is a single stranded DNA- and ATP-dependent helicase that has a role in chromosome translocation. This is a domain of unknown function C-terminal to its von Willebrand factor A domain, that also occurs in bacterial hypothetical proteins.
Ku_C: Ku70/Ku80 C-terminal arm. The Ku heterodimer contributes to genomic integrity through its ability to bind DNA double-strand breaks and facilitate repair by the non-homologous end-joining pathway. This is the C terminal arm. This alpha helical region embraces the beta-barrel domain Ku of the opposite subunit.
SAP: The SAP (after SAF-A/B, Acinus and PIAS) motif is a putative DNA binding domain found in diverse nuclear proteins involved in chromosomal organization.

(▬) segment of low compositional complexity

FIGURE 12 Domain organization of the autoantigens Ku80 and Ku70.

are believed to take part in the regulation of protein synthesis at the level of the protein elongation step. The P1/P2 protein complex binds to the 28S rRNA GTPase center though the N-terminal domain of the P0 ribosomal protein.

Autoantibodies to ribosomal P proteins (anti-P) are directed against the three ribosomal phosphoproteins (P0, P1, and P2). Their immunodominant epitope (a single sequential one) is contained within the 22 amino acid C-terminal peptide shared by the three P proteins.[133] This epitope has been associated with SLE and especially with central nervous system (CNS) involvement.[134] Using as substrate a synthetic 22-amino acid peptide corresponding to the ribosomal P0, P1, and P2 common epitope, we studied the specificity and sensitivity of the method and evaluated the frequency

Ribosomal_60s: 60s acidic ribosomal protein, component of ribosomes, the particles that catalyze mRNA-directed protein synthesis in all organisms.

FIGURE 13 Domain organization of the autoantigen ribosomal P.

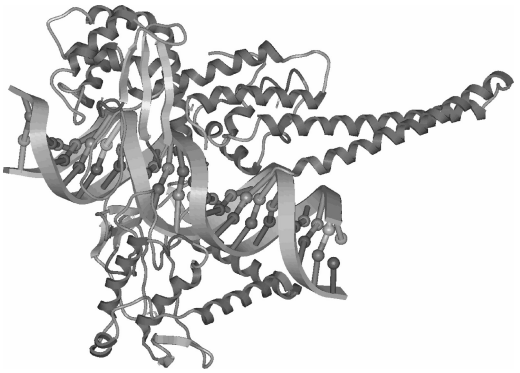
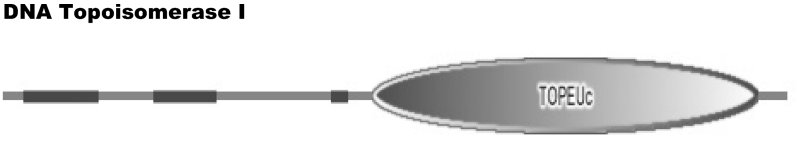


FIGURE 14 Structure of human DNA topoisomerase I in complex with a 22-base pair DNA duplex (PDB: 1A36).

and clinical associations of anti-P antibodies in two groups of SLE patients: (a) unselected patients, and (b) patients with CNS involvement. The overall prevalence of anti-ribosomal P antibodies in SLE patients with active CNS disease was significantly higher than in unselected SLE patients. These antibodies were found in a high proportion of patients without anti-cardiolipin antibodies (52.4%) and were associated with diffuse CNS involvement, such as psychiatric disorders (71%) or epilepsy (75%).[135]

K. Scleroderma 70 (Topoisomerase I)

The topoisomerase I or Scl-70 enzyme (Figures 14, 15) functions include breaking and rejoining DNA strands, which enables supercoiled DNA to unwind. Autoantibodies to topoisomerase I are markers for SSc. Epitope mapping studies based on topoisomerase I recombinant fragments revealed the major antigenic sites to be in the 405–484aa,[136] 453–560aa,[137]



TOPEUc: Eukaryotic-like DNA topoisomerase I, otherwise known as relaxing enzyme, untwisting enzyme or swivelase, is one of the two types of enzyme that catalyze the interconversion of topological DNA isomers and is vital for the processes of replication, transcription, and recombination. Topoisomerase I catalyses the ATP-independent breakage of single-stranded DNA, followed by passage and rejoining of another single-stranded DNA region. This reaction brings about the conversion of one topological DNA isomer into another: e.g., relaxation of positive and negative super-coils; interconversion of simple and knotted rings of single-stranded DNA; and intertwisting of single-stranded rings of complementary sequences.

(▬) segment of low compositional complexity

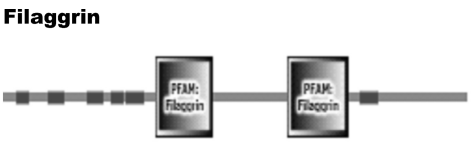
FIGURE 15 Domain organization of the autoantigen DNA topoisomerase I.

and 512–563aa[138] regions of the autoantigen. B-cell epitope mapping with synthetic 20-mer peptides (overlapping by eight residues) allowed identification of the epitopes at the peptide level.[139] Four major epitopes were found to react with anti-topoisomerase I sera, but not with the control sera: 205–224aa (epitope I), 349–368aa (epitope II), 397–416aa (epitope III), and 517–536aa (epitope IV). Epitopes II to IV are localized at highly exposed sites of the topoisomerase I tertiary structure, whereas epitope I is localized at a less accessible site. Epitope IV was also found to reside within the antigenic region previously defined in two out of three studies with recombinant fragments.[137,138] In a cohort of 81 SSc patients with early disease, patients with antibodies recognizing at least three of the four epitopes had 3.1 times the risk of developing pulmonary fibrosis compared with patients whose sera recognized no epitopes or only one or two of the four epitopes.[139]

L. Filaggrin

AKA and APF were originally identified in patients with RA. AKA and APF have similar antigenic specificities, recognizing filaggrin of buccal epithelial cells[41,42] (Figure 16). Filaggrin is a cytokeratin filament aggregating protein of the epidermis that plays a structural role in upper cornified cells. This protein is subsequently proteolyzed into free amino acids.

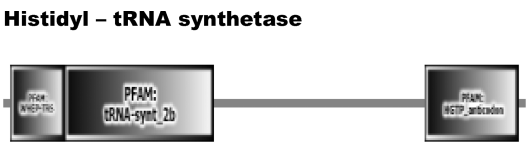
The main target structures in filaggrin have been identified in sequences containing citrulline, an arginine residue post-translationally modified by the enzyme peptidylarginine deiminase.[43,44] The major epitope has been identified in the 306–324aa region. These modified residues have been shown to form the central position of epitopes targeted by anti-filaggrin antibodies. In addition, recombinant filaggrin fragments were recognized by RA autoantibodies only after *in vitro* enzymatic deimination.[43] The antibodies bound to the citrullinated substrates were also detected, using several synthetic peptides containing citrulline, with a high disease specificity in over 80% of RA sera.



Filaggrin: Filaggrins are filament-associated proteins that interact with keratin intermediate filaments of terminally differentiating mammalian epidermis via disulphide bond formation The proteins are synthesised as large, insoluble, highly-phosphorylated precursors, containing multiple tandem repeats of 324 amino acids, which are not separated by a large linker. The precursor is deposited as keratohyalin granules. During terminal differentiation, it is dephosphorylated and proteolytically cleaved.

(▬) segment of low compositional complexity

FIGURE 16 Domain organization of the autoantigen filaggrin.



WHEP-TRS domain: A conserved domain of 46 amino acids, called WHEP-TRS has been shown to exist in a number of higher eukaryote aminoacyl-transfer RNA synthetases. This domain could contain a central α-helical region and may play a role in the association of tRNA-synthetases into multienzyme complexes.
tRNA-synt_2b: tRNA synthetase class II core domain. This domain is the core catalytic domain of tRNA synthetases and includes glycyl, histidyl, prolyl, seryl and threonyl tRNA synthetases. A tRNA synthetase catalyses the attachment of an amino acid to its cognate transfer RNA molecule in a highly specific two-step reaction.
HGTP_anticodon: Anticodon binding domain. This domain is found in histidyl, glycyl, threonyl and prolyl tRNA synthetases; it is probably the anticodon binding domain.

FIGURE 17 Domain organization of the autoantigen Jo-1.

M. t-RNA Synthetase (Jo-1)

The Jo-1 antigen (Figure 17) is identical to the histidyl tRNA synthetase enzyme that binds histidine to its cognate transfer RNA ($tRNA^{his}$) so that the amino acid can be incorporated into a growing polypeptide chain. The existence of autoantibodies targeting the Jo-1 autoantigen is a diagnostic marker for autoimmune myositis (PM/DM) and a prognostic marker for disease severity.[54] A major epitope has been described in the amino-terminal portion (60 amino acids) of the molecule in a protein region that has a coiled-coil three-dimensional structure (WHEP-TRS domain).[140,141]

N. The Autoantigen PM/Scl-100

The human analogue of the yeast exosome, the PM/Scl particle, which consists of 11–16 polypeptides, has exoribonuclease activity during RNA processing.[142] The function of these proteins has not been identified. Anti-PM/Scl-100 antibodies are seen in 50% of patients with the clinical overlap syndrome of PM-SSc. The PM/Scl-100 protein (Figure 18) is the prime target of autoimmune response against the PM/Scl complex. Immune response against anti-PM/Scl-100 was shown to be predominately directed against a 15-amino acid region with α-helical secondary structure (231–245aa).[143]

O. The Autoantigen Proteinase 3

Autoimmune small-vessel vasculitis is strongly associated with the presence of ANCA. In the context of autoimmune vasculitis, ANCA with a cytoplasmic staining pattern by indirect immunofluorescence (c-ANCA) are generally directed against proteinase 3 (PR3) (Figure 19).[144] Antibodies to PR3 predominate in patients with Wegener's granulomatosis. These antibodies can interfere with the proteolytic activity of PR3.[145]

FIGURE 18 Domain organization of the autoantigen PM/Scl-100.

PR3, a serine proteinase of 29–32 kDa (Figure 20), is a highly-folded protein with four disulphide bridges keeping its 3-dimensional structure intact.[146] PR3 is processed into a mature form consisting of 222 amino acids.[147] Williams *et al.* identified several antigenic sites on PR3 that seemed to be exposed on the outside of the molecule. Some of these regions are close to the active site of PR3; one epitope even resides exactly on the active site.[148] In another study using linear peptides covering the entire sequence of PR3, four epitope areas were identified that were recognized preferentially by Wegener's granulomatosis sera drawn at initial presentation of disease. Two of these epitope areas were located near the active center of PR3.[149] Finally, Griffith *et al.* identified five regions on PR3 that were bound by PR3-ANCA, four of which were located near the catalytic site.[150] From the above-mentioned studies on epitope mapping of PR3-ANCA, it can be concluded

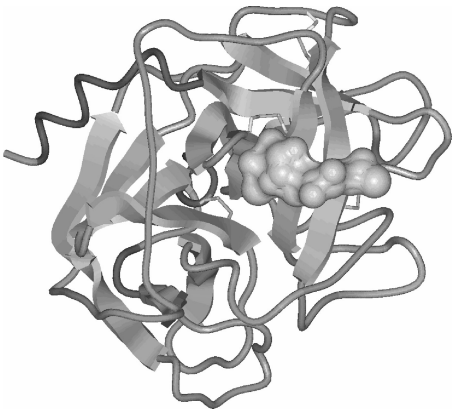


FIGURE 19 Structure of the complex of human proteinase 3, (in ribbon representation) with the third domain of the turkey ovomucoid inhibitor (in spacefill representation) (PDB: 1FUJ).

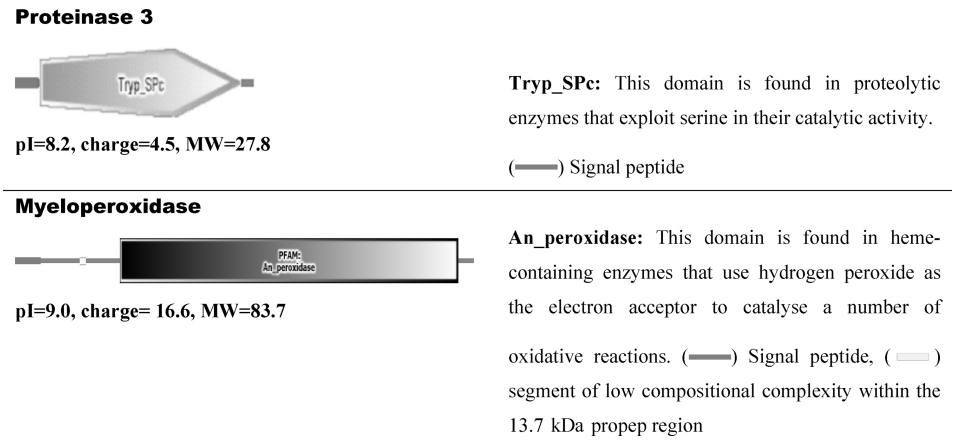


FIGURE 20 Domain organization of the autoantigens proteinase 3 and myeloperoxidase.

that PR3-ANCA from patients with Wegener's granulomatosis, especially in their first presentation, recognize a restricted number of immunodominant epitopes. Binding of these epitopes by PR3-ANCA is expected to have functional consequences for the molecule.

P. The Myeloperoxidase Autoantigen

In the context of autoimmune small-vessels vasculitis, ANCA with a perinuclear staining pattern by indirect immunofluorescence (p- ANCA) are mainly directed against MPO (Figures 20, 21).[58] Antibodies to MPO predominate in patients with Churg-Strauss syndrome and microscopic polyarteritis.[59]

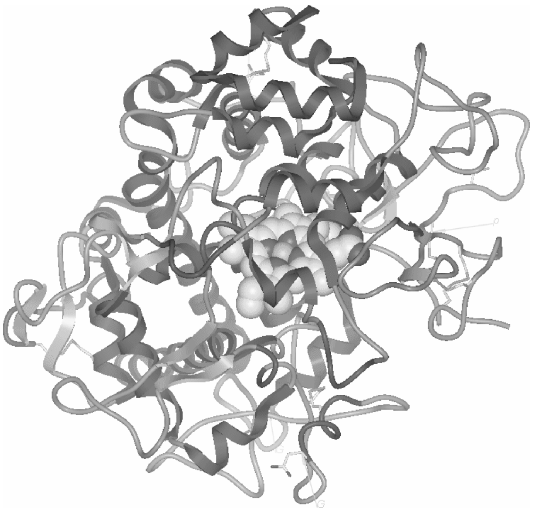


FIGURE 21 Structure of the human myeloperoxidase (in ribbon representation). The heme ring is depicted in spacefill representation (PDB: 1D2V).

MPO is localized in azurophilic granules of neutrophils and lysosomes of monocytes. It is highly cationic and moves toward negatively-charged nuclear membrane when ethanol disrupts the granule membranes of ethanol-fixed neutrophils. Therefore, the perinuclear pattern observed by indirect immunofluorescence microscopy in anti-MPO positive sera is caused by an artifact during the fixation of neutrophils.[151,152] MPO catalyzes H_2O_2-mediated peroxidation of the chloride ion to hypochlorous acid,[153] which is effective in killing phagocytized bacteria and viruses.

Structurally, MPO is a 120–150 kDa dimer of heterodimers. Each heterodimer is composed of one heavy (55–60 kDa) and one light (14–15 kDa) chain carrying one heme group. MPO contains mainly conformational epitopes.[154] Linear epitopes have also been reported in the heavy chain of the autoantigen.[155] Audrain *et al.* have shown that autoantibodies to human MPO are directed against at least four different epitopes.[156] Fujii *et al.* have reported that the most common antigenic sites are regions on the upstream side of Met341 and Met409 near the N-terminus of the MPO heavy chain as well as on a domain on the downstream side of Gly598 near the C-terminus.[157]

V. B-CELL EPITOPES OF AUTOANTIGENS AS TOOLS TO INVESTIGATE THE AUTOIMMUNE RESPONSE

A. Complementary Epitopes & Anti-Idiotypic Antibodies

The idiotypic network theory was proposed by the 1984 Nobel laureate Niels Jerne.[158] He hypothesized that antibodies can act as antigens and elicit anti-antibodies (called anti-idiotypic antibodies). If anti-idiotypic antibodies target the antigen-binding sites of the idiotypic antibodies, then they can either (a) compete with the antigen for the same binding site (Ab2β anti-idiotypic antibodies according to Jerne's classification) or (b) elicit anti-anti-idiotypic antibodies with similar antigenic specificity with the idiotypic antibodies.[159] Thus, anti-idiotypic antibodies can either "neutralize" idiotypic antibodies or elicit antibodies with the same antigenic specificity as they have. In fact, under these conditions, an anti-idiotypic network is established that regulates the production of idiotypic antibodies.

Based on the detailed knowledge of the antigenic structures that are recognized by autoantibodies, one can design complementary epitopes that are expected to be recognized by anti-idiotypic antibodies, using the "molecular recognition" theory.[160] According to this theory, 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. Interestingly, many experimental data suggest that these interacting peptides have the ability to generate and detect interacting pairs of idiotypic and anti-idiotypic antibodies.[161]

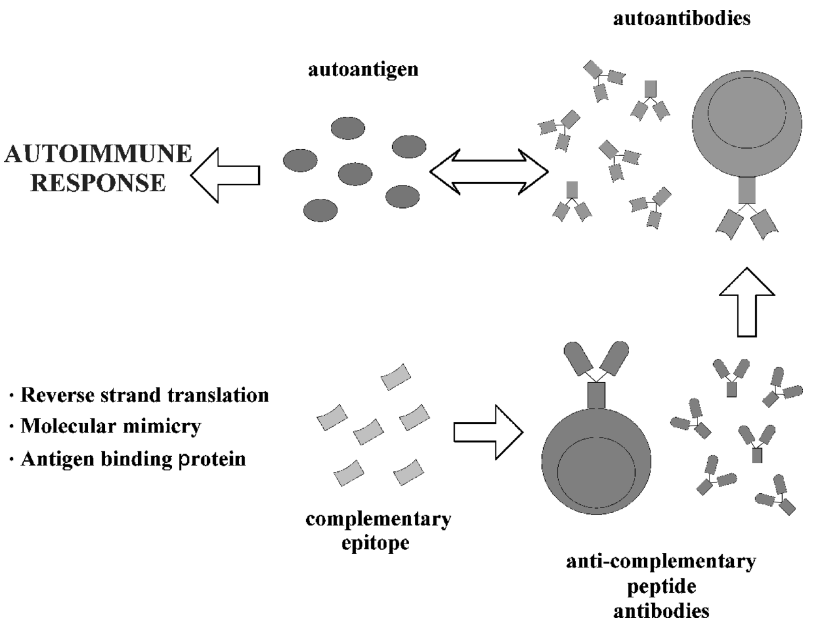


FIGURE 22 A proposed mechanism for the develepoment of an autoimmune response through complementary epitopes and anti-idiotypic antibodies. A complementary epitope (derived from antisense strand translation) or a complementary epitope mimic initiates the production of anti-complementary epitope antibodies that, in turn, elicit an anti-idiotypic response. The resultant antibodies react with the autoantigen, which is complementary to the initiating antigen, producing an autoimmune response.

Recent findings indicate that autoimmunity can be initiated through an immune response against a peptide that is complementary to the autoantigen[162] (Figure 22). Pedergraft and coworkers demonstrated that a subset of PR3-ANCA patients harbors antibodies directed against the translated protein product of the middle fragment (105–201aa) of the antisense RNA of PR3, termed complementary PR3 or cPR3.[163] These antibodies were not present in patients with anti-MPO autoantibodies (MPO-ANCA), SLE patients, or healthy controls. It was also demonstrated that human anti-cPR3 and anti-PR3 antibodies are an idiotypic–anti-idiotypic pair, that mice immunized with cPR3 develop anti-cPR3 and anti-human PR3 antibodies, and that complementary PR3 transcripts are present in peripheral leukocyte RNA from a subset of ANCA patients.[162,163]

Recent studies in our laboratory have demonstrated that in SLE and SS there is also an active idiotypic–anti-idiotypic network targeting the two major B-cell epitopes of La/SSB and their complementary peptides.[164] The anti-idiotypic antibodies were isolated using the complementary epitopes and were found to bind anti-La/SSB antibodies, competing with La/SSB epitopes for their antigen binding site. In some cases, these anti-idiotypic antibodies were capable of completely masking anti-La/SSB antibodies, abolishing their anti-La/SSB reactivity. A specific procedure, developed with the use of

complementary peptides for the release of anti-La/SSB antibodies from their anti-idiotypic counterpart, was applied in 44 anti-La (−), anti-Ro/ANA (+) sera from patients with SLE and SS. Ninety-four % of SS sera and 80% of SLE sera were found to be negative for anti-epitope 349–364aa antibodies prior to the treatment with complementary epitope. After the treatment, all SS and SLE sera became positive for anti-epitope 349–364aa antibodies, while none of the normal sera exhibited a positive reaction. Heating without addition of complementary epitope 349–364aa had no effect on patient sera reactivity. Thus, virtually all anti-Ro/ANA (+) sera possess hidden anti-La/SSB antibodies that can be unmasked by treatment with the complementary epitope. Animal studies also demonstrated that mice immunized with complementary epitopes of La/SSB develop anti-human La/SSB antibodies.[165] Thus, the complementary epitopes of La/SSB appear to have the potential to induce an autoimmune response against La/SSB autoantigen.

B. Early Epitope Recognition in Autoimmune Diseases and Epitope Spreading

The initiation of autoimmunity occurs when 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 analyzing cellular and biochemical processes have led to an extensive body of literature that characterizes human autoimmune diseases on multiple levels. Nevertheless, the precise etiology of most human autoimmune diseases remains largely unexplained, and the initiating immunogens are unclear.

In SLE, the onset and progression of autoantibody development before clinical diagnosis has been studied. Arbuckle *et al.*, using the U.S. Department of Defense Serum Repository with over 30 million specimens prospectively collected from 5 million U.S. Armed Forces personnel, evaluated serum samples obtained from 130 persons before they received a diagnosis of SLE.[166] 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, APL, anti-Ro, and anti-La antibodies were present earlier than anti-Sm and anti-snRNP antibodies. Anti-dsDNA antibodies were found later than ANA and earlier than anti-snRNP antibodies. The earliest autoantibodies detected in the preclinical period, as individuals progressed toward clinical SLE, were antibodies to Ro60 (mean 3.7 years before the disease onset). McClain *et al.* mapped the initial, predisease target of the anti-Ro60 autoantibody response to the region, 169–180aa (TKYKQRNGWSHK), of the autoantigen.[167] This region belongs to the SLE related 169–190aa epitope, previously identified by Routsias *et al.*[95] (Figure 23).

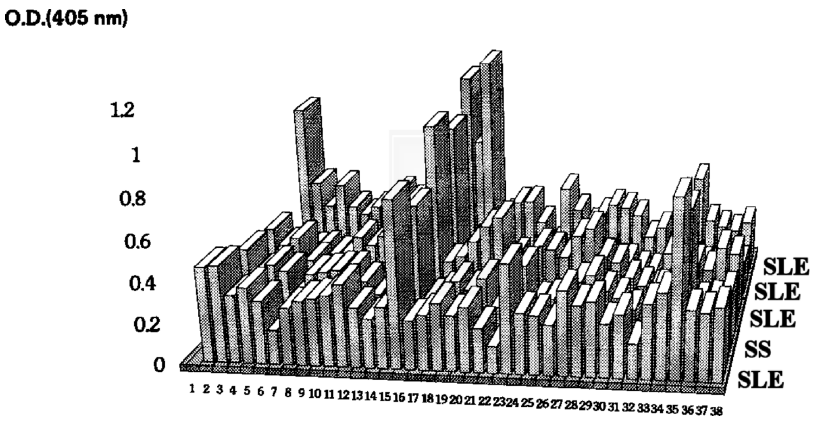


FIGURE 23 Epitope mapping of the Ro60 autoantigen using overlapping synthetic peptides revealed that the major epitope, recognized by SLE sera, is located in the 169–190aa region.[95]

This 169–180aa epitope was found by McClain and coworkers to cross-react with a peptide (GGSGSGPRHRDGVRR, 58–72aa) from the latent viral protein Epstein-Barr virus nuclear antigen-1 (EBNA-1).[167] Notably, no areas of primary sequence homology exist between EBNA-1 58–72aa and Ro169–180aa although these peptides have similarly high isoelectric points (12.0 and 10.5, respectively). However, animals immunized with either the 169–180, epitope of Ro60 or the cross-reactive EBNA-1 epitope progressively developed autoantibodies binding multiple epitopes of Ro and spliceosomal autoantigens. These animals eventually developed some of the clinical symptoms of lupus, such as leukopenia, thrombocytopenia, and renal dysfunction. Although these experiments indicate a cross-reaction between the initial epitope of Ro60 and the 58–72aa region of EBNA-1, any involvement of Epstein Barr virus in the pathogenesis of SLE has to be proved. In addition, other possible cross-reactions of Ro60 169–180aa epitope with various xenoantigens have to be studied (e.g., the Ro60 169–176aa, TKYKQRNG, with that of L-polymerase 53–60aa, TKYKIRNG of human parainfluenza virus 1).

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 sites on an autoantigen.[168] This process is not a feature restricted to systemic autoimmune diseases but is a common characteristic of the natural immune response mounted against some pathogens. In this regard, McClain *et al.* showed that immunization 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.[169] 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 Monneaux *et al.*[170,171] 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 recognized by CD4+ T cells from lupus mice and is often targeted by autoantibodies very early during the course of the disease.[170,172] 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 also be considered as important "initiator" sequences, *e.g.*, the recurring proline-rich sequence, PPPGMRPP, present in several snRNPs or the dimethylarginine-modified CRG repeats present on the B, D1, and D3 autoantigens.[121]

C. Epitopes and Post-Translational Modifications

The majority of mammalian proteins have PTMs, which potentially can be recognized by the immune system as self neoepitopes. PTMs are either driven by enzymes or occur spontaneously, but the extent of protein modification varies in inflammatory and autoimmune disorders. These associations have been known for some time, but their effects on disease etiology are still unclear. Two amino acid modifications have been described as targets of systemic autoimmunity (Figure 24).

1. Arginine Modifications. Arginine residues are susceptible to three forms of modification: methylation, deimination, and citrullination. Two of them have been correlated with systemic autoimmunity.

a. 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 symmetric dimethylarginine residues.[63,173] 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

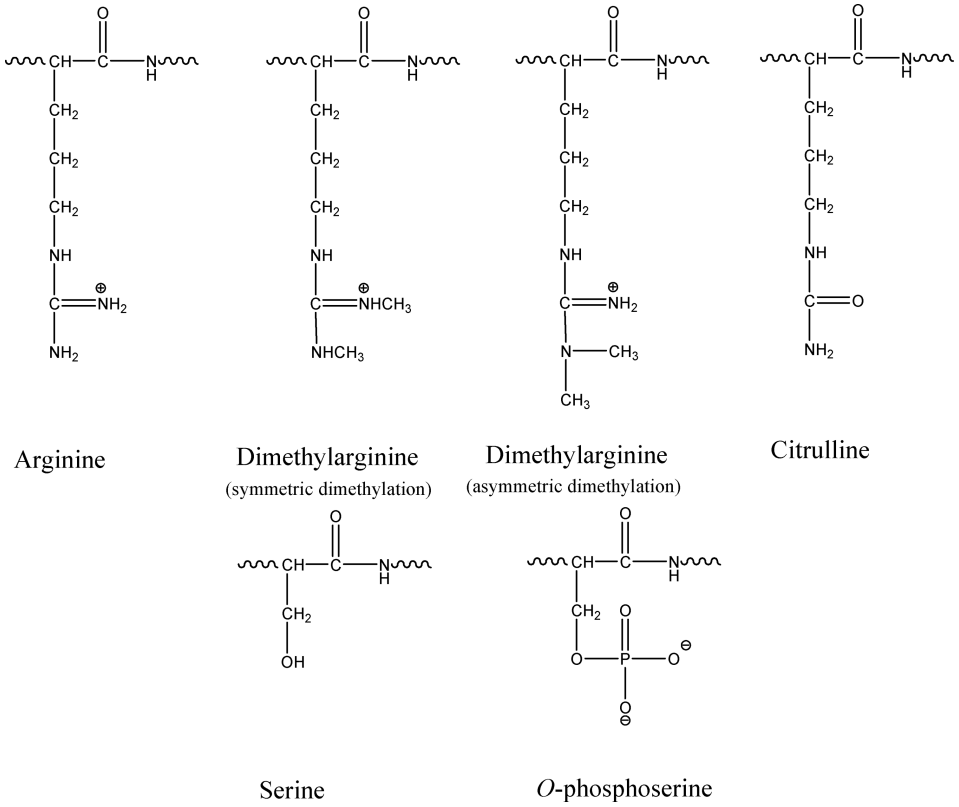


FIGURE 24 PTMs of arginine and serine residues. Note that citrullination of arginine removes a positive charge from the protein while phosphorylation of serine adds two negative charges to the molecule.

detecting a subclass of anti-Sm antibodies by ELISA.[174] Thus, symmetrical dimethylarginine residues act as targets for autoantibodies in SLE. It was recently shown that the same autoantigens contain asymmetric dimethylarginine in addition to the already-reported symmetric dimethylarginine residues.[175] The effect of this modification in autoantibody binding has not been studied yet.

b. 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 RA.[176] One of the major autoantigens in RA, filaggrin, is citrullinated by PAD and provides several targets for autoantibody binding. Schellekens *et al.*, using a selected set of citrullinated peptides, developed an ELISA with a sensitivity of more than 70% and an impressive specificity of better than 96%.[43] The same investigators developed a new diagnostic test for sera using a cyclic citrullinated peptide (CCP).[177] Using RA and non-RA sera, the anti-CCP ELISA proved to be highly specific (98%), with a

reasonable sensitivity (68%).[177] In comparison to the IgM-RF ELISA, the anti-CCP ELISA had 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. Combinating the anti-CCP and the IgM-RF ELISAs resulted in a significantly higher positive predictive value of 91% and a slightly lower negative predictive value of 78% as compared to the IgM-RF ELISA alone.[178] Similarly, when anti-CCP and RF antibodies were combined, the specificity exceeded 99%.[179] Finally, follow-up studies revealed that anti-CCP antibodies could be detected in early stages of RA and that these antibodies were associated with more severe radiological damage.[180,181] Thus, the detection of anti-CCP antibodies has several advantages over the classical APF and AKA assays and may replace them in the near future.

2. 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.

a. Spliceosomal Antigens. Phosphorylated components of U1 snRNP particles are specifically recognized by autoantibodies of SLE patients and CD4+ T-cells from lupus-prone mice (MRL/lpr mice). Monneaux *et al.* demonstrated the importance for antigenicity of phosphorylation on a specific serine residue of U1-70K in MRL/lpr mice.[182] Previously, these investigators had identified an epitope at residues 131–150 of the U1-70K protein that was recognized by antibodies as well as by CD4+ T cells in two strains of lupus mice.[183] They subsequently synthesized 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 $Ser^{140}P$, but not the peptide with $Ser^{137}P$, was recognized by antibodies as well as by CD4+ T cells.

b. 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 5′-terminal oligopyrimidine (5′TOP).[184] Thus, La exists in distinct states that differ in subcellular localization and is associated RNAs, which can be discriminated by serine 366 phosphorylation. This specific phosphorylation resides within an antigenic determinant of La/SSB. This major B-cell epitope was previously identified in our laboratory and was found to reside in the 349–368aa region of La/SSB.[85] Our studies indicate that the antigenicity of the epitope is significantly enhanced upon phosphorylation of serine 366 (unpublished data).

VI. FUTURE DIRECTIONS

The extensive study of B-cell epitopes of intracellular autoantigens provide 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:

A. Autoantibody screening test methodologies can be improved using large-scale arrays with specific autoantigen epitopes. These arrays are able to perform large-scale multiplex characterizations of autoantibody responses against structurally diverse autoantigens.[185]
B. Chemical modifications of autoepitopes 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.[11]
C. Complementary peptides can efficiently neutralize 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 autoimmune response. In this regard, foreign epitopes, 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 initial epitope to others can be utilized 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.

VII. 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 neo-epitope formation due

to PTMs of autoantigens. Finally, some B-cell epitopes appear to be promising diagnostic markers for the evaluation of systemic autoimmune disorders.

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