
Review Article

B-Cell Epitopes of Intracellular Autoantigens: Myth and Reality

N. M. Moutsopoulos¹, J. G. Routsias², P. G. Vlachoyiannopoulos²,
A. G. Tzioufas², and H. M. Moutsopoulos²

¹ Oral Infection and Immunity Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Athens, Greece ² Department of Pathophysiology, Medical School, University of Athens-Greece, Athens, Greece

Communicated by A. Papavassiliou. Accepted November 1, 1999.

Introduction

Patients with systemic autoimmune diseases often develop autoantibodies directed against large intracellular complexes composed of a number of proteins that are non-covalently associated with nucleic acid components. For example, antibodies against nucleosomes, a complex of double stranded DNA and histones, are typically found in Systemic Lupus Erythematosus (SLE) patients. Antibodies targeting spliceosomes, the complex of small nuclear RNAs (snRNAs) or uridine rich RNAs (URNAs) with (Sm) and (RNP) proteins involved in the processing of RNA, are found in SLE and Mixed Connective Tissue Disease (MCTD) patients. Similarly, antibodies against the Ro ribonucleoprotein complex that consist of Ro and La proteins bound to small cytoplasmic RNAs (YRNAs) are most commonly detected in primary Sjogren's Syndrome (pSS) and SLE; whereas, antibodies to topoisomerase I, an enzyme involved in the DNA duplication process, are found in patients with Systemic Scleroderma (SCL) (1,2).

Despite the heterogeneity of systemic autoimmune diseases, the autoantibody response against these complexes shares common characteristics. First, the response is antigen-driven, so the antibodies are immunoglobulin

G (IgG) in class and undergo affinity maturation (3,4). Second, autoantibodies usually exist in linked sets. For example, anti-La antibodies almost always coexist with anti-Ro antibodies in SS sera, while the anti-Sm autoantibody usually accompanies anti-U₁RNP in SLE sera (5). Third, humoral autoimmunity is primarily directed against the protein components of ribonucleoprotein particles (6).

The autoantibody profile constitutes a useful tool for the diagnosis of autoimmune diseases and a prognostic indicator for disease evolution. Several studies over the last decade have attempted to identify the antigenic determinants (epitopes) of autoantibodies (B-cell epitopes) (7,8). B-cell epitopes can be either linear, formed by adjacent amino acid residues in the primary structure of a protein, or conformational, formed by amino acid residues from distant regions in the sequence of a given protein that are spatially juxtaposed upon folding (9). Epitopes are characterized as major when they are predominantly recognized by the majority of a patient's sera and minor when the patient's antibodies less frequently recognize them.

Methods for B-cell Epitope Identification

Diverse approaches have been used to map B-cell epitopes. For several reasons (eg., the presentation of antigenic fragments, the type of assay employed, and the selection and number of

Address correspondence and reprint requests to: Prof. H. M. Moutsopoulos, Dept. of Pathophysiology, School of Medicine, National University of Athens-Greece, 75.M.Asias St. 115 27, Athens, Greece. Phone: 0030-1-7771095; Fax: 0030-1-7703876; E-mail: hmoutsop@dh.uoa.gr

patient sera), conflicting results have arisen (10). It is well documented that no individual approach currently applied to identify B-cell epitopes in a protein is capable of complete mapping of epitopes in a given protein. Therefore, several authors have emphasized that results of B-cell epitope mapping studies, obtained by different methods, may be considered complementary to each other and not as exclusive (10,11).

A first method that is used is the proteolytic digestion of affinity-purified autoantigen, followed by testing of the fragments against autoantibodies. A second method includes the preparation of recombinant fragments of the autoantigen and testing of autoantibodies against them. Finally, a third method is the synthesis of multiple overlapping peptides covering the whole sequence of the autoantigen in order to determine the immunoreactive peptides and the minimum required length for autoantibody binding.

Proteolytic digestion of the antigen cannot be used for detailed epitope mapping, as the fragments of the antigen are usually large (15-35 kD), and the whole technique is rather laborious (10).

The testing of affinity-purified or recombinant autoantigen can reveal both linear and conformational epitopes, but are unable to define either the exact localization or the exact length of the epitope. Furthermore the antigenicity of the recombinant protein may be partly different from the native one, due to post-translational modifications (glycosylations /phosphorylations) that may occur in the native protein (10).

Evaluation of B-cell epitopes using synthetic peptides reveals primarily linear epitopes. Nevertheless, if the targeting peptide is long enough (exceeding 10-12 amino acid residues), useful information on putative conformational epitopes can be achieved. This method can detect amino acid residues, critical for the binding of the autoantibody (12).

Discrepancies among laboratories can also exist, due to the length of the fragments that are chosen for testing. The use of longer peptides does not necessarily lead to a higher level of reactivity, but usually leads to a higher level of specificity. Shorter peptides can mimic linear immunogenic determinants, but might also fold easily into the proper orientation required for the binding of a low affinity—not a specific antibody. In contrast, longer peptides do not easily possess the conformational freedom to fit in to the binding groove of a non-specific antibody. Furthermore, longer peptides can fold and pre-

sent conformational epitopes made up from chemical groups or electrostatic charges located in different parts of its amino acid chain.

Many studies have shown that the antigenic reactivity of polypeptides highly depends on the assay used (13). In some cases, a free soluble polypeptide is more active; whereas, in others, antibodies react preferentially with immobilized peptides adsorbed to a solid phase or conjugated to a carrier (14). Sakarellos-Daitsiotis et al. (15) showed that anchoring of peptides to sequential oligopeptide carrier (SOCn) induces a favorable arrangement of the conjugated peptides, so that potent antigens have the proper stereochemical orientation for interaction with antibody. The literature concerning the fine specificity of the autoimmune response is very controversial, since investigations using different sera and assays concluded in B-cells recognizing different epitopes (16).

Several studies directly assessed the nature of the antibody response against the native versus recombinant 60kDRo. Itoh and Reichlin (14) showed that 23% of the anti-Ro sera bind only native antigen. McCauliffe et al. (17), using recombinant 60kDRo fragments, found that a number of sera, positive by immunodiffusion failed to react with denatured recombinant protein in Western blots analysis, indicating the importance of native structure for some anti-Ro sera. It is interesting to note that these sera usually belong to SS patients, in contrast to SLE sera, which possess common anti-Ro antibodies against the denatured form of the antigen (18).

Wahren et al. (19), using recombinant Ro60 proteins, identified a major antigenic domain located in the middle part of the protein (181-320) that was recognized by 86% of the sera. Two further antigenic domains were found in the N- and C-terminal ends of the protein that were recognized by 20% of the sera. Similar results were obtained by McCauliffe et al. using recombinant fragments of Ro60kD antigen (20). These investigators identified a major epitope within residues 139-326aa and a minor one in the C-terminal region (aa 410-538). The localization of the main Ro60 epitope on the central part of the molecule was further confirmed by two more studies (21,22). In the first study, Saitta et al. (21) performed epitope mapping of Ro 60kD with recombinant fragments and found that the most frequently recognized region was located between residues 155 and 295.

Epitope mapping with recombinant polypeptides gave us valuable information about the antigenic site(s) of Ro60 protein, but the exact location of the antigenic determinants were revealed only after the application of synthetic peptides (Fig. 1). Wahren et al. (23) identified a major epitope in synthetic peptide 216-245. Routsias et al. (24) performed an extensive and analytical epitope mapping of the same protein and found that a major antigenic region, recognized mainly by SS sera, resided in residues 216-232; whereas, a major epitope, recognized mainly by SLE sera, was located in the 175-184 region of the antigen. Finally, Scofield and associates, using synthetic octapeptides, found a number of epitopes covering the entire length of Ro60 (25-29). One of the peptides (485-492) shares sequence similarity with the N-protein of Vesicular Stomatitis Virus (29). However, the population of anti-Ro60 antibodies directed against the above-mentioned region seemed to be rather limited (30).

Research on 52-kd Ro unraveled the advantages and disadvantages of the assays used for B-cell epitope mapping. Several studies that used recombinant-truncated proteins and synthetic peptides (31-37) identified a common epitope overlapping the leucine zipper motif (spanning residues 183-232; Fig. 2). However, Ricchiuti et al. (38), by use of overlapping (by three amino acids) synthetic peptides, did not identify any epitope within the leucine zipper motif. Pourmand et al. (39) recently showed, using recombinant fragments

of Ro52, that the zinc finger region contained two conformation-dependent epitopes, detectable under reducing conditions in immunoblotting.

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), LaC (amino acids 111-242) and LaL2/3 (amino acids 246-408) (40). Later, more detailed and analytical epitope mapping suggested antigenic determinants in various parts of the La antigen (Fig. 3; [41-46]). To a greater extent than the Ro60kD and Ro52kD epitopes, La epitopes reside in functional parts of the protein. Such antigenic determinants seem to exist within the La-RNP motif (35,36,41) and the La-ATP binding site (39). However, autoantibodies against the RNP motif are capable of binding La, even if the RNP motif is utilized for binding to human cytoplasmic (hY)RNA in the full RoRNP particle (41), whereas antibodies against the ATP binding site are unable to react with La when ATP is bound on the protein (44).

Why Study B-cell Epitopes of Autoantigens?

An initial reason to study B-cell epitopes is to develop assays for autoantibody detection with higher sensitivity and specificity. The existing

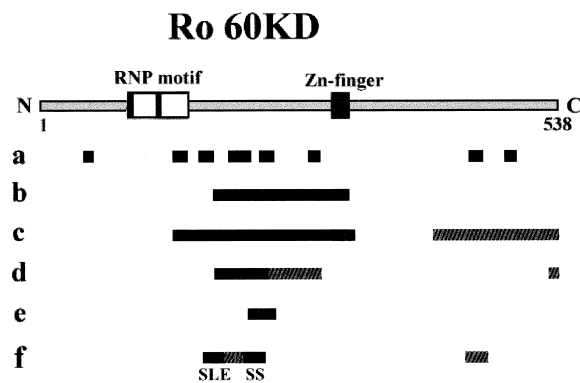


Fig. 1. Schematic representation of autoepitopes of the Ro60kD protein. a. Scofield and associates (25-29), b. Wahren et al. (19), c. McCauliffe et al. (17), d. Veldhoven et al. (22), e. Wahren et al. (23) and f. Routsias et al. (24). Minor epitopes are shown in gray.

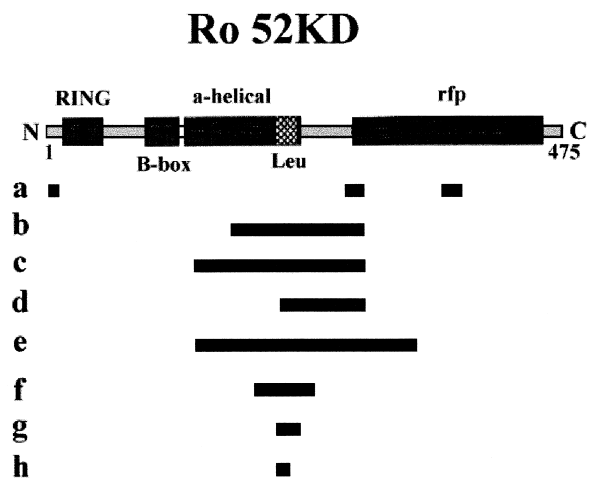


Fig. 2. Schematic representation of autoepitopes of the Ro52kD protein. a. Ricchiuti et al. (38), b. Byuon et al. (32), c. Blange et al. (33), d. Bozic et al. (31), e. Dorner et al. (34), f. Dorner et al. (35), g. Kato et al. (36) and, h. Frank et al. (37).

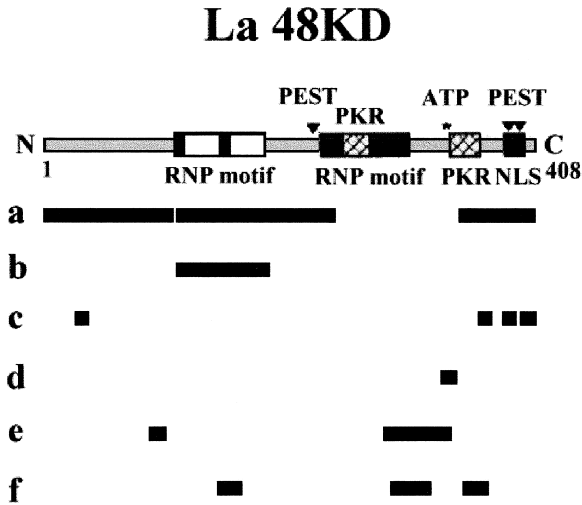


Fig. 3. Schematic representation of autoepitopes of the La48kD protein. a. McNeilage et al. (40,41), b. Rischmueller et al. (42), c. Haaheim et al. (43), d. Troster et al. (44), e. Kohsaka et al. (45), and f. Tzioufas et al. (46). Abbreviations: NLS, nuclear localization signal; PEST, Pro-Glu-Ser-Thr; PKR, domain homologous to RNA-dependent protein kinase.

autoantibody assays rely on recombinant or affinity-purified human autoantigens, which result in increased cost and low stability of the assays. Chemically synthesized epitope peptide analogues can offer an alternative source of a low-cost antigen of high purity and stability. Accordingly, Yiannaki et al. (47) investigated the use of large synthetic peptides as epitope analogues, as well as peptides attached to sequential oligopeptide carriers (SOC) as substrates, and developed a peptide-based ELISA and dot blot protocol for the detection of anti-La/SSB antibodies. They compared the (349-364) SOC4 peptide-based assay with assays based on recombinant native La protein, La C-terminal and N-terminal with a mutation at base pair 640. Of the anti-La sera tested, 88.1% were reactive with both the synthetic peptide (349-364) SOC4 and the recombinant La protein, 83% were reactive with the La N terminus, and 67.8% of sera were reactive with the C terminus. Using sera that were anti-Ro positive but anti-La negative, 37% were reactive with the recombinant protein, 26% with the La N-terminus, and only 11% with the synthetic protein. These results suggest that the synthetic epitope analogues exhibit high sensitivity and specificity for the detection of anti-La antibodies in ELISA and dot blot techniques.

The use of small peptides was tested in previous studies (16). The failure of binding of anti-La/SSB human positive sera to synthetic La peptides reported previously was probably due to an incomplete survey of sequential epitopes, excluding peptides that we found to be reactive. The four larger antigenic La peptides used by Yiannaki et al. (47) proved to be very sensitive and highly specific using the ELISA and dot blot. Petrovas et al. (48) developed a sensitive and highly reproducible solid-phase enzyme immunoassay (ELISA) to show that five copies of the synthetic heptapeptide PPGMRPP—a major epitope of the Sm autoantigen—anchored in five copies to a sequential oligopeptide carrier (SOC), [(PPGMRPP)₅-SOC₅] was a suitable antigenic substrate to identify anti-Sm antibodies.

A second important reason to study B-cell epitopes is to establish the association of autoantibodies with the clinical picture of a given disease. One has to keep in mind though, that the nature of the immunogen is an important factor in determining the autoimmune response. Therefore, the molecular characteristics of the autoantigen may determine its clinical relevance. Different antigen processing and presentation of the immunogen may result in T-cell responses to different epitopes, which may induce autoantibodies with different specificities that may lead to a distinct clinical picture.

A classic example is the association of autoantibodies to ribosomal P proteins (anti-P antibodies) that react preferentially with a common epitope consisting of 22 amino acid residues located at the carboxyl-terminal end of all P proteins. These autoantibodies, when found in SLE patients, have been correlated with neuropsychiatric lupus (49). Tzioufas et al. (50) developed an ELISA using a synthetic 22mer peptide substrate, corresponding to the common epitope of P proteins. According to their results, the clinical significance of the presence of anti-P antibodies in the general lupus clinic was rather limited. On the other hand, their significance in patients with active central nervous system (CNS) involvement was very useful, since it could discriminate among lupus psychiatric disorders and epilepsy, as well as vascular disease not associated with anticardiolipin antibodies.

Another example of autoantibody specificity associated with clinical complications is that of anti-topo I antibodies in systemic sclerosis. Rizou et al. (51) identified four major

epitopes, spanning the sequences 205-224, 349-368, 397-416, and 517-536, that were strongly associated with the risk of developing Pulmonary Fibrosis (PIF), a serious complication in SSn patients. In fact, the magnitude of the association of anti-topo antibodies with pulmonary fibrosis varied in different studies. Some investigators found a strong association, while others did not (52). Rizou et al. (51) suggested that, although many patients with aSSc had anti-topo I antibodies, PIF developed in the patients with antibodies reacting with more than two of the above-defined epitopes. The same study also revealed that, although the above epitopes were linear, they were very closely associated in terms of tertiary structure, in a way that they constituted large conformational epitopes on the other surface of the molecule, according to the classical "paradigm" of molecular immunology.

Additional interest in B-cell epitope research concerns the study of antigenic structures recognized by autoantibodies. Research on the structure of autoantigens may lead to important information about their function. Studying the details which govern the interaction of antigen with autoantibody may contribute to the understanding of the immune response that heralds autoimmunity.

The study of structures within the autoantigen molecules has disclosed homologous sequences shared between B-cell epitopes of autoantigens and foreign proteins. These homologous primary sequences have putative 3D-structures capable of reacting with the same antibody molecule (usually with different affinity). This phenomenon is called "mol-

ecular mimicry." Examples of molecular mimicry include: the close relationship among antigenic octapeptides of 60kDa Ro and the N-protein of Vesicular Stomatitis Virus (25), the C-terminal sequence of SmD sharing broad homology with EBNA-I, a nuclear antigen whose synthesis is induced by Epstein Barr virus (55), and several other autoantigens with sequence similarity to viral proteins (Table 1). These results led to the hypothesis that foreign antigens (viral in particular) may cause an autoimmune reaction. Nevertheless, not all individuals infected with the specific viruses have autoimmune reactions. It appears that only those individuals that are at risk to develop an autoimmune disorder have an uncommon immune response towards these viruses.

Besides the similarity with foreign molecules, the B-cell epitopes of autoantigens also disclosed a significant sequence homology with endogenous native structures. Routsias et al. (63) studied the biochemical and molecular characteristics of ¹⁶⁹TKYKQRNGWSHKDLLR-SHLKP¹⁹⁰ and ²¹¹ELYKEKALSVEKLLKY-LEAV²³² sequences (two major epitopes of the Ro60KD antigen), recognized by sera from SLE and pSS patients, respectively. Their study revealed a similar molecular conformation (as defined by circular dichroism and molecular modeling), as well as antigenicity between the HLA-DR3 β chain and the 169-190 Ro60KD epitope. This finding is particularly interesting since the autoimmune response directed towards Ro/ssa and La/ssb autoantigen is highly associated with this particular HLA class II alloantigen. Thus, autoantibodies reacting with such exposed regions of the major histocom-

Table 1. Sequence similarity between viral proteins and human autoantigen epitopes

Autoantigen	Viral Antigen	Reference(s)
Ro/SSA 60kD	Vesicular Stomatitis Virus N-protein	25, 29, 30
	Adenovirus Transcription Factor	24
La/SSB 48kD	Herpes Virus Group Proteins	53
Sm D 13kD	Epstein Barr Proteins (EBNA-2, EBNA-1)	54, 55
Sm B/B' 26kD/27kD	Epstein Barr Protein (EBNA-1)	56
	HIV p24 gag protein	57, 58
U1 RNP 70kD	HIV gp120/41 envelope complex	59
	Retroviral p30gag protein	60
U1RNP-C 18.5kD	Herpes Simplex Virus type I protein (ICP4)	61
DNA Topoisomerase I 110kD	Retroviral p30gag protein	62

patibility complex (MHC)-II are capable to activate B cells or macrophages through dimerization and cross-linking of these molecules (64,65). We should also note that dimerization / polymerization of surface MHC-II molecules is the mechanism by which specific superantigens act (66,67).

Finally, and potentially most importantly, studying the epitopes of autoantigens may be helpful for understanding the mechanism through which autoimmune responses are triggered and autoantibodies are generated. Mechanisms of autoimmune response remain obscure and seem to be regulated by a combination of multiple factors. Considerable evidence suggests that autoimmune diseases occur in genetically susceptible individuals. Specific disease-associated alleles have been characterized (for example, the MHC HLA class II loci in Type I Diabetes) and high-risk genotypes (68). Nevertheless, the disease-associated genotypes are commonly found in healthy individuals and no particular gene is necessary or sufficient for disease expression.

A phenomenon described as "epitope spreading" was recently described in SLE patients, and may give insight into some of the mechanisms involved in the evolution of autoimmunity. Early in the disease, autoantibodies were found to bind proline-rich motifs (PPGMRPP and PPPGIRGP). With time, however, the autoantibody response expanded to neighboring epitopes in a progression that was reproducible from one patient to the next (50). This phenomenon has been observed in several animal models as well. Rabbits immunized with the peptide PPGMRPP initially develop an antibody response to the peptide. Over time, the recognized epitope spreads to other areas of the protein from which the immunogenic peptide was derived (69).

Epitope spreading also was shown in rabbits immunized with antigenic and non-antigenic regions of the 60kD Ro. After boosting, the rabbits developed an expanded immune response to many different regions of 60kD Ro, many of which were very similar to the common epitopes of human anti-Ro sera (28). In addition, spreading of autoimmunity towards the whole Ro/La RNP complex occurred, as indicated by the appearance of anti-La antibodies. When the same investigators attempted to reproduce this experiment in mice, they observed that immunization of an animal with a human 60kD Ro peptide resulted in epitope

spreading only if the peptides were highly homologous to the corresponding murine Ro60. If the degree of homology was low, as was the case for the Ro epitope 413-428, then the immune response was limited to the human sequence-derived immunogen (70). Other examples of such animal models are: mice immunized with antigenic histone peptides that developed high titers of anti-single stranded DNA and nucleosome antibodies (71); specific strains of mice that, after immunization with the peptide DWEYSVWLFSN (a sequence of the anti-double stranded (ds) DNA antibody R4A), developed anti-ds DNA antibodies; animals immunized with a 13 to 15 amino acid peptide from ovarian zona pellucida (ZP3—the primary sperm receptor) that developed autoimmune oophoritis; and specific inbred mice strains which, after immunization with central nervous system proteins, developed experimental allergic encephalomyelitis (EAE) (72).

Of particular interest is the relationship between molecular mimicry and epitope spreading. Molecular mimicry could represent a special issue of intermolecular epitope spreading, thereby bypassing many of the usual control elements and maturation steps of the primary immune response. In this regard, the autoimmune response might involve both molecular mimicry, as a triggering factor, followed by epitope spreading. James and colleagues (27) immunized rabbits with a peptide from Epstein-Barr virus Nuclear Antigen-1, possessing sequence similarity with the PPGMRPP Sm B/B' common epitope, and observed typical lupus anti-spiceosomal autoimmunity, suggesting coexistence of molecular mimicry and epitope spreading mechanisms.

Several studies outline the differences between autoantibody responses against Ro and La autoantigens in different diseases, such as SS and SLE. First, autoantibodies in SLE are directed mainly against Ro autoantigen (30–40%) and much less against La (10–15%) (73); whereas, in SS, both anti-Ro and anti-La autoantibodies coexist with increased frequency (70–80%) (74). Second, SLE autoantibodies target primarily the denatured form of Ro60, but SS sera usually recognize the natural form of the same autoantigen (18,31). Thus, it's not surprising that epitope mapping of Ro and La autoantigens with synthetic peptides disclosed disease-specific epitopes (24,46). In the case of SS sera, Ro and La epitopes reside away from

the RNP motifs (which are utilized for particle assembly in full Ro/La RNP), in contrast to SLE sera epitopes, which lie either within the RNP motif (in La protein) or nearby (in Ro60 protein; Fig. 4). These differences in the fine specificity of the epitopes might be due to different mechanisms operating at the onset of the disease (before epitope spreading occurs). So it is possible that SS autoantibodies are directed against full (functional) Ro/La RNP complexes, whereas SLE antibodies target individual Ro/La RNP components.

An additional question concerning the nature of the autoimmune response is the pathogenicity of autoantibodies. It is difficult to explain pathogenicity of autoantibodies against intracellular antigens. Growing evidence suggests that antibodies can penetrate into living cells, in some cases by means of surface-exposed proteins. Furthermore, a number of studies have demonstrated that intracellular antigens can be expressed on the surface membrane of the cell undergoing apoptosis (75–79). Interestingly, viral-induced apoptosis results in the co-clustering of autoantigens and viral antigens in small surface blebs of apoptotic cells (80). Specific complexes of viral and self-antigens encountered in this setting may provide a unique challenge to brake tolerance.

Animal models are also useful for determining the pathogenicity of autoantibodies. Serum antibodies to a variety of nuclear con-

stituents occur in SLE and are implicated in the pathophysiology of the disease via immune complex-mediated injury. In an effort to determine pathogenicity of autoantibodies and relate it to epitope spreading, Vlachoyi-annopoulos et al. (81) immunized rabbits with the sequence PPGMRPP (a major B-cell epitope of the Sm autoantigen) anchored to a sequential oligopeptide carrier (SOC) that had been proven to increase the immunogenicity of peptides. High titers of anti-(PPGMRPP)₅-SOC₅ antibodies occurred in all the animals immunized with this particular peptide. However, in contrast to previous findings (56), antibodies recognizing the Sm antigen or precipitating the native structures of snRNPs did not appear in the sera of the immunized animals. Nevertheless, the immune response induced by (PPGMRPP)₅-SOC₅ was associated with immune-mediated kidney injury that involved diffuse and segmental increase of mesangial matrix cells, crescent formation and segmental glomerular necrosis.

Reality and Myth

Identification of the B-cell epitopes of autoantigens has led to useful information regarding the structural and functional characteristics of the antigenic determinants that are targeted. Epitopes that are prominently targeted in

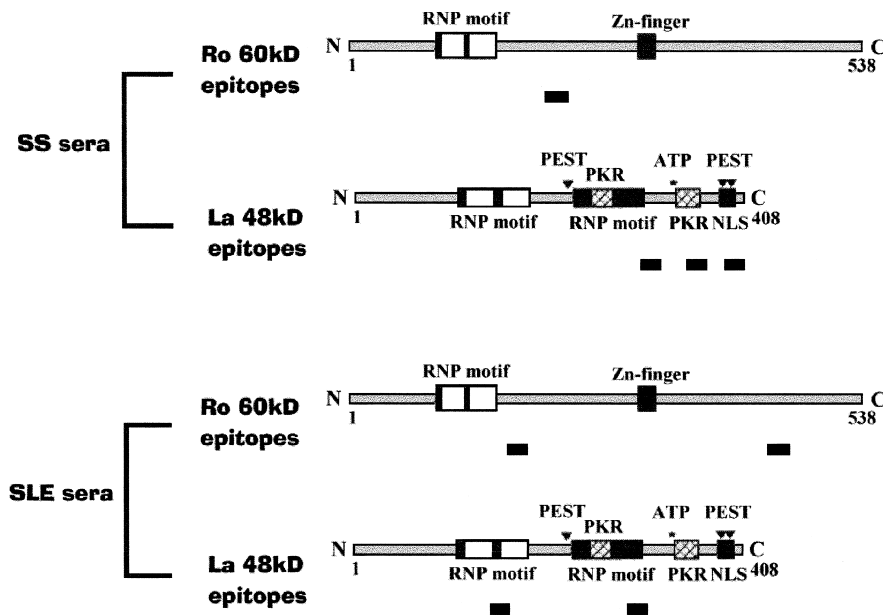


Fig. 4. Disease specific epitopes on autoantigens Ro60kD and La48kD (24,26).

specific diseases (major epitopes) have been characterized and serve as diagnostic markers of disease and disease progression. Assays for autoantibody detection with high specificity and sensitivity have been produced and associations of autoantibodies with the clinical picture of a disease have been achieved in many cases. These findings constitute the recent reality in the field of B-cell epitope research. Part of this reality is the understanding of the autoimmune triggering mechanism, which contributes to the generation of autoantibodies.

Many mechanisms have been proposed to explain the presence of autoantibodies. These include polyclonal B-cell activation, cross-reaction with foreign antigens (molecular mimicry), idiotype-antiidiotype response, activation of anergic autoreactive lymphocyte clones, or direct recognition of the autoantigen by the immune system.

Different mechanisms have also been proposed for the diversity of the autoantibody response that has been described in autoimmunity. They include inter- and intra-molecular epitope spreading (69). Intermolecular spreading may be due to the generation of distinct populations of autoantibodies to conformational epitopes shared in varying degrees among autoantigens. Another more likely possibility is that intra- and inter-molecular spreading could occur because helper signals are provided by a non-tolerant T_{helper}-cell clone to clonally distinct B-cells targeting different components of the RNP particle (82). Spreading also has been explained as an anti-hapten-type response. After the initial triggering reaction, specific autoantibodies are generated toward a whole particle or complex. A change in one component might lead to an anti-hapten-like response directed to associated macromolecules (83).

There is a great deal to be learned about mechanisms of autoimmunity and studying B-cell epitopes can be a useful tool. There is a lot more to be learned about B-cell epitopes and this knowledge could be useful for the design of refined diagnostic tools that would facilitate diagnosis and prognosis of disease. To achieve this knowledge, one must characterize the antigens that contain major or disease-associated epitopes. To study and characterize additional epitopes, improvement has to be achieved in the study of conformational epitopes. After the tertiary structure of the antigens and the epi-

topes is solved by crystallography or other means, studies for discontinuous epitopes can begin. Furthermore, T-cell epitopes also need to be studied and their impact in disease mechanisms needs to be investigated, since T-cell responses play a fundamental role in certain autoimmune mechanisms such as epitope spreading and autoreactive T cells are implicated in several autoimmune diseases (84).

Increased understanding in these areas will eventually lead to improved diagnosis and, more importantly, treatment of patients. It is speculated that epitope analogues could be used to block or modulate the autoimmune response. Possible methods might include treatment with tolerogenic doses of the major epitopes in order to induce anergy, or treatment with peptide antagonists (pseudomimetics) to block the receptors or complementary peptides to induce anti-idiotypic response. The use of B-cell epitopes to block the autoimmune response will remain a myth until proven otherwise.

References

1. Van Venrooij WJ. (1992) Autoantigens in connective tissue diseases. *Immun. Con. Tis. Dis.* **22**: 305-334.
2. Pollard KM, Reimer G, Tan EM. (1989) Autoantigens in scleroderma. *Clin. Exp. Rheumatol.* **7 (Suppl 3)**: S57-62.
3. Cohen PL, Cheek RL, Hadler JA, et al. (1987) The subclass distribution of human IgG rheumatoid factor. *J. Immunol.* **139**: 1466-1471.
4. Miller FW, Twitty SA, Biswas T, et al. (1985) Origin and regulation of a disease-specific autoantibody response. Antigenic epitopes, spectrotypic stability, and isotype restriction of anti-Jo-1 autoantibodies. *J. Clin. Invest.* **85**: 468-475.
5. Radar MD, Codding C, Reichlin M. (1989) Differences in the fine specificity of anti-Ro(SS-A) in relation to the presence of other precipitating autoantibodies. *Arthr. Rheum.* **32**: 1563-1571.
6. Plotz PH. (1990) What drives autoantibodies? The evidence from spontaneous human autoimmune diseases, in de Vries RRP, Cohen IR, van Rood JJ. (eds) *The Role of Micro-Organisms in Non-Infectious Diseases*. Springer-Verlag, London, pp. 111-121.
7. Elkon KB. (1992) Use of synthetic peptides for the detection and quantification of autoantibodies. *Mol. Biol. Rep.* **16**: 207-212.
8. Wahren M, Solomin L, Pettersson I and Isenberg D. (1996) Autoantibody repertoire to Ro/SSA and La/SSB antigens in patients with primary and secondary Sjogren's syndrome. *J. Autoimmun.* **9**: 537-544.

9. Geysen HM, Rodda SJ, Mason TJ. (1986) The delineation of peptides able to mimic assembled epitopes. In Porter R, Whelan J. (eds) *Synthetic Peptides as Antigens*. London, Pit man, pp 130–149.
10. Pettersson I. (1992) Methods of epitope mapping. *Mol. Biol. Rep.* **16**: 149–153.
11. Morris-GE (1998) Epitope mapping. *Methods Mol. Biol.* **80**: 161–172.
12. Geysen HM, Rodda SJ, Mason TJ, Tribbick G and Schoofs PG. (1987) Strategies for epitope analysis using peptide synthesis. *J. Immunol. Meth.* **102**: 259–274.
13. Dietzgen R and Zaitlin M. (1991) Alleged common antigenic determinant of tobacco mosaic virus coat protein and the host protein ribulose-1,5-biphosphate carboxylase is an artifact of indirect ELISA and western blotting. *Virology* **184**: 397–398.
14. Itoh Y, Reishlin M. (1992) Autoantibodies to the Ro/SSA antigen are conformation dependent. II. Anti-60 kD antibodies are mainly directed to the native protein; anti-52 kD antibodies are mainly directed to the denatured protein. *Autoimmunity* **14**: 57–65.
15. Sakarellos-Daitsiotis M, Tsikaris V, Sakarellos C, Vlahogiannopoulos PG, Tzioufas AG, Moutsopoulos HM. (1999) Peptide carrier: a new helicoid-type sequential oligopeptide carrier (SOCn) for developing potent antigens and immunogens. *Methods in Enzymology* **19**: 133–141.
16. Scofield RH, Farris DA, Horsfall AC and Harley JB. (1999) Fine specificity of the autoimmune response to the Ro/SSA and La/SSB ribonucleoproteins. *Arthr. Rheum.* **42**: 199–209.
17. McCauliffe DP, Yin H, Wang L-X, Lucas L. (1994) Autoimmune sera react with multiple epitopes on recombinant 52 and 60 kDa Ro(SSA) proteins. *J. Rheumatol.* **21**: 1073–1080.
18. Tszaka K, Fujii T, Akizuki M, et al. (1994) Clinical significance of antibodies to native or denatured 60-kd or 52-kd Ro/SS-A proteins in Sjogren's syndrome. *Arthritis Rheum.* **37**: 88–92.
19. Wahren M, Ruden U, Andersson B, Ringertz NR, Pettersson I. (1992) Identification of antigenic regions of the human Ro 60 kD protein using recombinant antigen and synthetic peptides. *J. Autoimmun.* **5**: 19–32.
20. McCauliffe DP, Yin H, Wang LX, et al. (1994) Autoimmune sera react with multiple epitopes on recombinant 52 and 60 kDa Ro(SSA) proteins. *J. Rheumatol.* **21**: 1073–1080.
21. Saitta MR, Arnett FC, Keene JD. (1994) 60-kDa Ro protein autoepitopes identified using recombinant polypeptides. *J. Immunol.* **152**: 4192–4202.
22. Veldhoven CH, Pruijn GJ, Meilof JF, et al. (1995) Characterization of murine monoclonal antibodies against 60-kD Ro/SS-A and La/SS-B autoantigens. *Clin. Exp. Immunol.* **101**: 45–54.
23. Wahren M, Muller S and Isenberg D. (1999) Analysis of B-cell epitopes of the Ro/SS-A autoantigen. *Immunology Today* **20**: 234–239.
24. Routsias JG, Tzioufas AG, Sakarellos DM, et al. (1996) Epitope mapping of the Ro/SSA 60KD autoantigen reveals disease-specific antibody-binding profiles. *Eur. J. Clin. Invest.* **26**: 514–521.
25. Scofield RH, Harley JB. (1991) Autoantigenicity of Ro/SSA antigen is related to a nucleocapsid protein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. U.S.A.* **88**: 3343–3347.
26. Huang SC, Yu H, Scofield RH, et al. (1995) Human anti-Ro autoantibodies bind peptides accessible to the surface of the native Ro autoantigen. *Scand. J. Immunol.* **41**: 220–228.
27. James JA, Scofield RH, Harley JB. (1997) Lupus humoral autoimmunity after short peptide immunization. *Ann. N. Y. Acad. Sci.* **815**: 124–127.
28. Scofield RH, Henry WE, Kurien BT, et al. (1996) Immunization with short peptides from the sequence of the systemic lupus erythematosus-associated 60-kDa Ro autoantigen results in anti-Ro ribonucleoprotein autoimmunity. *J. Immunol.* **156**: 4059–4066.
29. Scofield RH, Dickey WD, Jackson KW, James JA, Harley JB. (1991) A common autoepitope near the carboxyl terminus of the 60kD Ro ribonucleoprotein: sequence similarity with a viral protein. *J. Clin. Immunol.* **11**: 378–388.
30. Routsias JG, Sakarellos-Daitsiotis M, Detsikas E, et al. (1994) Antibodies to EYRKK vesicular stomatitis virus-related peptide account only for a minority of anti-60KD antibodies. *Clin. Exp. Immunol.* **98**: 414–418.
31. Bozic B, Pruijn GJ, Rozman B, et al. (1993) Sera from patients with rheumatic diseases recognize different epitope regions on the 52-kD Ro/SS-A protein. *Clin. Exp. Immunol.* **94**: 227–235.
32. Byuon JP, Slade SG, Reveille JD, Hamel JC, Chan EKL. (1994) Autoantibody responses to the “native” 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjogren's syndrome. *J. Immunol.* **152**: 3675–3684.
33. Blange I, Ringertz NR, Pettersson I. (1994) Identification of antigenic regions of the human 52kD Ro/SS-A protein recognized by patient sera. *J. Autoimmun.* **7**: 263–274.
34. Dorner T, Feist E, Wagenmann A, et al. (1996) Anti-52 kDa Ro(SSA) autoantibodies in different autoimmune diseases preferentially recognize epitopes on the central region of the antigen. *J. Rheumatol.* **23**: 462–468.
35. Dorner T, Feist E, Held C, et al. (1996) Differential recognition of the 52-kd Ro(SS-A) antigen by sera from patients with primary biliary cirrhosis and primary Sjogren's syndrome. *Hepatology* **24**: 1404–1407.
36. Kato T, Sasakawa H, Suzuki S, et al. (1995) Autoepitopes of the 52-kd SS-A/Ro molecule. *Arthr. Rheum.* **38**: 990–998.

37. Frank MB, Itoh K, McCubbin V. (1994) Epitope mapping of the 52-kD Ro/SSA autoantigen. *Clin. Exp. Immunol.* **95**: 390–396.
38. Ricchiuti V, Briand JP, Meyer O, Isenberg DA, Pruijn G, Muller S. (1994) Epitope mapping with synthetic peptides of 52-kD SSA/Ro protein reveals heterogenous antibody profiles in human autoimmune sera. *Clin. Exp. Immunol.* **95**: 397–407.
39. Pourmand N, Pettersson I. (1998) The Zn²⁺ binding domain of the human Ro 52 kDa protein is a target for conformation dependent autoantibodies. *J. Autoimmun.* **11**: 11–17.
40. McNeilage LJ, Macmillan EM, Whittingham SF. (1990) Mapping of epitopes on the La(SS-B) autoantigen of primary Sjogren's syndrome: identification of a cross-reactive epitope. *J. Immunol.* **145**: 3829–3835.
41. Weng YM, McNeilage J, Topfer F, et al. (1993) Identification of a human-specific epitope in a conserved region of the La/SS-B autoantigen. *J. Clin. Invest.* **92**: 1104–1108.
42. Rischmueller M, McNeilage LJ, McCluskey J, et al. (1995) Human autoantibodies directed against the RNA recognition motif of La (SS-B) bind to a conformational epitope present on the intact La (SS-B)/Ro (SS-A) ribonucleoprotein particle. *Clin. Exp. Immunol.* **101**: 39–44.
43. Haaheim LR, Kvakestad R, et al. (1993) Immunodominant epitopes on the La/SSB autoantigen recognized by sera from patients with primary Sjogren syndrome and systemic lupus erythematosus. Sjogren's Syndrome: State of the Art-4th International Symposium, Tokyo, Japan, pp. 11–13.
44. Troster H, Bartsch H, Klein R, et al. (1995) Activation of a murine autoreactive B cell by immunization with human recombinant autoantigen La/SS-B: characterization of the autoepitope. *J. Autoimmun.* **8**: 825–842.
45. Kohsaka H, Yamamoto K, Fujii H, et al. (1990) Fine epitope mapping of the human SS-B/La protein. Identification of a distinct autoepitope homologous to a viral gag polyprotein. *J. Clin. Invest.* **85**: 1566–1574.
46. Tzioufas AG, Yiannaki E, Sakarellos DM, Routisias JG, et al. (1997) Fine specificity of autoantibodies to La/SSB: epitope mapping, and characterization. *Clin. Exp. Immunol.* **108**: 191–198.
47. Yiannaki EE, Tzioufas AG, Bachmann M, Hantoumi J, Tsikaris V, Sakarellos-Daitsiotis M, Moutsopoulos HM. (1998) The value of synthetic linear epitope analogues if La/SSB; specificity, sensitivity and comparison of methods. *Clin. Exp. Immunol.* **112**: 152–158.
48. Petrovas CJ, Vlahoyiannopoulos PG, Tzioufas AG, Alexopoulos C, Tsikaris V, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM. (1998) A major Sm epitope anchored to sequential oligopeptide carriers is a suitable antigenic substrate to detect anti-Sm antibodies. *J. Immunol. Meth.* **220**: 59–68.
49. The LS, Isenberg DA. (1994) Antiribosomal P protein antibodies in systemic lupus erythematosus. A reappraisal. *Arthr. Rheum.* **37**: 307–315.
50. Tzioufas AG, Tzortzakis NG, Panou-Pomonis E, Boki KA, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM. (2000) The clinical relevance of antibodies to ribosomal-P common epitope in two targeter SLE populations: a large cohort of the consecutive patients and patients with active CNS disease. *Ann. Rheum. Dis.* **59**: 99–104.
51. Rizou C, Ioannidis JPA, Panou-Pomonis E, Sakarellos-Daitsiotis M, Sakarellos C, Moutsopoulos HM. (2000) B-cell epitope mapping of DNA topoisomerase I defines epitopes strongly associated with pulmonary fibrosis in systemic sclerosis. *Am. J. Resp. Cell Mol. Biol.* **22**: 344–351.
52. Kuwana M, Kaburaki J, Mimori T, Tojo T, Homma M. (1993) Autoantigenic epitopes on DNA topoisomerase I. Clinical and immunogenetic associations in systemic sclerosis. *Arthr. Rheum.* **36**: 1406–1413.
53. Haaheim LR, Halse AK, Kvakestad R, Stern B, Normann O, Jonsson R. (1996) Serum antibodies from patients with primary Sjogren's syndrome and systemic lupus erythematosus recognize multiple epitopes on the La(SS-B) autoantigen resembling viral protein sequences. *Scand. J. Immunol.* **43**: 115–121.
54. Incaprera M, Rindi L, Bazzichi A, Garzelli C. (1998) Potential role of the Epstein-Barr virus in systemic lupus erythematosus autoimmunity. *Clin. Exp. Rheumatol.* **16**: 289–294.
55. Sabbatini A, Bombardieri S, Migliorini P. (1993) Antibodies from patients with systemic lupus erythematosus bind a shared sequence of SmD and Epstein-Barr virus-encoded nuclear antigen EBNA 1. *Eur. J. Immunol.* **23**: 1146–1152.
56. James JA, Gross T, Scofield RH, et al. (1995) Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B'-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. *J. Exp. Med.* **181**: 453–461.
57. De Keyser F, Hoch SO, Takei M, Dang H, De Keyser H, Rokeach LA, Talal N. (1992) Cross-reactivity of the B/B' subunit of the Sm ribonucleoprotein autoantigen with proline-rich polypeptides. *Clin. Immunol. Immunopathol.* **62**: 285–290.
58. Talal N, Garry RF, Schur PH, Alexander S, Dauphinee MJ, Livas IH, et al. (1990) A conserved idotype and antibodies to retroviral proteins in systemic lupus erythematosus. *J. Clin. Invest.* **85**: 1866–1871.
59. Douvas A, Takehana Y, Ehresmann G, Chernyovskiy T, Daar ES. (1996) Neutralization of HIV type 1 infectivity by serum antibodies from a

- subset of autoimmune patients with mixed connective tissue disease. *AIDS Res. Hum. Retroviruses* **12**: 1509–1517.
60. Query CC, Keene JD. (1987) A human autoimmune protein associated with U1 RNA contains a regional homology that is cross-reactive with retroviral p30gag antigen. *Cell* **51**: 211–220.
 61. Misaki Y, Yamamoto K, Yanagi K, Miura H, Ichijo H, Kato T, Mato T, Welling-Wester S, Nishioka K, Ito K. (1993) B cell epitope on the U1 snRNP-C autoantigen contains a sequence similar to that of the herpes simplex virus protein. *Eur. J. Immunol.* **23**: 1064–71.
 62. Maul GG, Jimenez SA, Riggs E, Ziemnicka-Kotula D. (1989) Determination of an epitope of the diffuse systemic sclerosis marker antigen DNA topoisomerase I: sequence similarity with retroviral p30gag protein suggests a possible cause for autoimmunity in systemic sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **86**: 8492–8496.
 63. Routsias JG, Sakarellos-Daitsiotis M, Sakarellos C, et al. (1998) Structural, molecular and immunological properties of linear B-cell epitopes of Ro60KD autoantigen. *Scand. J. Immunol.* **47**: 280–287.
 64. Kaye J, Janeway-CA J. (1984) The Fab fragment of a directly activating monoclonal antibody that precipitates a disulfide-linked heterodimer from a helper T cell clone blocks activation by either allogeneic Ia or antigen and self-Ia. *J. Exp. Med.* **159**: 1397–1412.
 65. Cambier JC, Lehmann KR. (1989) Ia-mediated signal transduction leads to proliferation of primed B lymphocytes. *J. Exp. Med.* **170**: 877–886.
 66. Bernatchez C, Al DR, Mayer PE, et al. (1997) Functional analysis of Mycoplasma arthritidis-derived mitogen interactions with class II molecules. *Infect. Immun.* **65**: 2000–2005.
 67. Roussel A, Anderson BF, Baker HM, et al. (1997) Crystal structure of the streptococcal superantigen SPE-C: dimerization and zinc binding suggest a novel mode of interaction with MHC class II molecules. *Nat. Struct. Biol.* **4**: 635–643.
 68. Todd JA. From genes to aetiology in a multifactorial disease, type 1 diabetes. (1999) *Bioessays* **21**: 164–174.
 69. James JA, Harley JB. (1998) B-cell epitope spreading in autoimmunity. *Immunol. Rev.* **164**: 185–200.
 70. Scofield RH, Kaufman MK, Baber U, et al. (1999) Immunization of mice with human 60kD Ro peptides results in epitope spreading if the peptides are highly homologous between human and mouse. *Arthr. Rheum.* **42**: 1017–1024.
 71. Kaliyapermal A, Mohan C, Wu W, Datta SK. (1996) Nucleosomal peptide epitopes for nephritis inducing T helper cells of murine lupus. *J. Exp. Med.* **183**: 2459–2469.
 72. Caponi L. (1996) The cross reactivity of autoantibodies in connective tissue diseases. *Clin. Exp. Rheumatol.* **14**: 425–431.
 73. van Venroij W, Charles P, Maini RN. (1991) The consensus workshops for the detection of autoantibodies to intracellular antigens in rheumatic diseases. *J. Immunol. Meth.* **140**: 181–189.
 74. Reichlin M: Antibodies to RNA proteins in Systemic Lupus Erythematosus. (1991) The significance of paired immune responses. In Talal N. (ed): *Molecular Autoimmunity*. Academic Press, New York, pp 51–63.
 75. Igarashi T, Itoh Y, Fukunaga Y, et al. (1995) Stress-induced cell surface expression and antigenic alteration of the Ro/SSA autoantigen. *Autoimmunity* **22**: 33–42.
 76. Zhu J. (1995) Cytomegalovirus infection induces expression of 60 KD/Ro antigen on human keratinocytes. *Lupus* **4**: 396–406.
 77. Furukawa F, Lyons MB, Lee LA, et al. (1988) Estradiol enhances binding to cultured human keratinocytes of antibodies specific for SS-A/Ro and SS-B/La. Another possible mechanism for estradiol influence of lupus erythematosus. *J. Immunol.* **141**: 1480–1488.
 78. Furukawa F, Kashiwara SM, Lyons MB, et al. (1990) Binding of antibodies to the extractable nuclear antigens SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): implications for the pathogenesis of photosensitive cutaneous lupus. *J. Invest. Dermatol.* **94**: 77–85.
 79. Casciola RL, Anhalt G, Rosen A. (1994) Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**: 1317–1330.
 80. Rosen A, Casciola-Rosen L, Ahern J. (1995) Novel packages of viral and self-antigens are generated during apoptosis. *J. Exp. Med.* **181**: 1557–1561.
 81. Vlachoyiannopoulos PG, Petrosas C, Tzioufas AG, Alexopoulos C, Tsikaris V, Guialis A, Nakopoulou L, Sakarellos-Daitsiotis M, Sakarellos C, Davaris P, Moutsopoulos HM. (2000) No evidence of epitope spreading after immunization with the major Sm epitope P-P-G-M-R-P-P anchored to sequential oligopeptide carriers. *J. Autoimmunity* **14**: 53–61.
 82. Gordon T, Topfer F, Keech C, et al. (1994) How does autoimmunity to La and Ro initiate and spread? *Autoimmunity* **18**: 87–92.
 83. Reeves WH, Satoh M. (1996) Features of autoantigens. *Mol. Biol. Rep.* **23**: 217–26.
 84. Feller DC, de la Cruz VF. (1991) Identifying antigenic T-cell sites. *Nature* **349**: 720–723.