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Προέλευση, αντιγονική ειδικότητα και ρύθμιση των αυτοαντισωμάτων που στρέφονται κατά του Ro/La RNP.

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32.
Origin, antigenic specificity and regulation of autoantibodies targeting Ro/La RNP

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Antibodies recognizing the Ro/La RNP particle are commonly found in a high proportion of sera from patients with systemic lupus erythematosus or Sjogren's syndrome. Although, the mechanism by which these autoantibodies arise is not known, their autoantigenic targets have been studied extensively. The Ro/La ribonucleoprotein complex (RNP) is formed by the noncovalent association of La and Ro60 autoantigens with a small cytoplasmic RNA (hYRNA) [1, 2]. Ro52 autoantigen is also transiently associated with Ro/La RNP [2]. Additional components of the complex have been recently identified as the proteins calreticulin [3] and nucleolin [4].

Epitope mapping with synthetic peptides, in our laboratory, revealed the precise antigenic regions of Ro60kD in 169–190 and 211–232 parts of the antigen [5]. One of them, the 169–190 epitope, was found to share conformational and antigenic similarity with HLADR3 β-chain. The homologous regions in these two proteins (HLA-DR3 β-chain and Ro60KD) were found to share similar molecular conformation (as defined by circular dichroism and molecular modeling), as well as common antigenic features [6]. 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 histocompatibility complex (MHC)-II are potentially capable to activate B cells or macrophages through dimerization and cross-linking of these molecules.

Although Ro60kd epitopes were identified as small peptidic moieties (22 aa in length) with rather limited reactivity against patient sera, their recognition by autoantibodies is conformation-dependent and their antigenicity is dramatically enhanced upon interaction with the molecular chaperone calreticulin [7]. Using complexes of highly purified human calreticulin with the linear epitopes of Ro60Kd, it was found that almost all positive anti-Ro60Kd sera bound strongly onto the newly formed conformation of the epitopes [7]. When calreticulin or the linear epitopes of Ro60Kd were tested individually with the same sera, the prevalence of positive reactions was much lower. In addition, sera from pSS or SLE

patients without anti-Ro/SSA antibodies did not react with the calreticulin–linear epitope complexes of Ro60Kd [7]. These observations suggest conformation-dependent enhancement of antigenicity of the Ro60Kd epitopes upon interaction with the chaperone protein calreticulin and such kind of complexes can potentially be used as substrates for the efficient detection of autoantibodies.

Recent studies in our laboratory have been also focused on the zinc finger motif of Ro60Kd protein. The zinc fingers are secondary structure elements, responsible for protein–DNA and protein–protein interactions. They can also hold putative conformational B-cell epitopes, since their structure is affected by zinc binding and redox conditions. Using synthetic peptide analogues corresponding to (i) to the zinc finger motif of Ro60Kd, spanning the region 301–327aa (Zif-1), (ii) a truncated form of the zinc finger motif, without the intermediate loop (310–319aa) of the molecule (Zif-2), and (iii) the intermediate loop of the zinc finger motif (Zif-3). It was found that the majority of anti-Ro/SSA and La/SSB positive sera from patients with pSS bound in 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 Ro52Kd, but not to autoantibodies [8]. Thus, different conformations of the zinc finger domain of Ro60kD, were employed in interaction with Ro52kD polypeptide and pSS autoantibodies.

B-cell epitope mapping of La/SSB was performed also in our laboratory using 20-mer synthetic peptides overlapping by eight amino acids covering the whole sequence of the protein. Peptides highly antigenic were those spanning the sequences: ^{147}HKAFKGSI^{154} (located within RRM motif: 113 – 182aa), ^{291}NGNLQLRNKEVT^{302}, ^{301}VTWEVLEGEVEKEALKKI^{318} and ^{349}GSGKGKVQFQGKKTKF^{364} [9]. The peptide-based ELISA assays, with the above described epitopes, presented sensitivities ranging from 78 to 90% and specificities from 69 to 94%. The most sensitive and specific peptide 349GSGKGKVQFQGKKTKF364 (>90% sensitivity and specificity) was synthesized in attachment with a tetramer sequential oligopeptide carrier SOC4 and used for immunoassay development. Ninety percent of anti-La positive sera were reactive with both the synthetic peptide 349–364aa and the recombinant La protein [10]. Thus, this epitope analogue exhibited comparable with the recombinant La/SSB value for the detection of anti-La/SSB antibodies. Clinical aspects of antibodies to linear B-cell epitopes of La/SSB in pSS were also studied by our group [11]. It was found that autoantibodies to the La/SSB epitope, p349–364aa, were significantly positively associated with longer disease duration, recurrent or permanent parotid gland enlargement, and a higher proportion of non-exocrine manifestations, compared to patients without autoantibodies [11].

Anti-idiotypic antibodies, reactive with idiotypes of autoantibodies, are capable of regulating the autoimmune response [12]. The same antibodies may also interfere in autoantibody detection by competing with antigen for binding in the same paratopic site (antigen inhibitable or Ab2h anti-idiotypic antibodies, according to Jerne's classification). In order to derive peptides capable of neutralizing anti-idiotypic antibodies, we have taken advantage of the antisense/complementary peptide approach [13]. This approach is based on the molecular recognition theory. According to this theory, the translation of two complementary mRNAs produces a pair of peptides with inverted hydrophobicity profiles that leads, under certain conditions, to strong interaction between these two (sense and antisense) peptides [14]. Interestingly, these peptides have the ability to generate interacting pairs of idiotypic and anti-idiotypic antibodies upon their application in animal immunizations [15]. In this regard, we prepared complementary peptides corresponding to major epitopes of La/SSB (289–308aa and 349–364aa) [13]. These peptides reacted with a significant proportion of patient sera with anti-La specificity. From these patients sera, anti-complementary epitope and anti-epitope antibodies were purified and digested with pepsin in order to produce $F(ab)_2$ fragments. The antibodies against epitopes found to specifically interact with the $F(ab)_2$ fragments of antibodies recognizing complementary epitopes and vice versa, suggesting their idiotype–anti-idiotype relation. Inhibition experiments demonstrated that anti-idiotypic antibodies compete with the antigen for the binding site (paratope) of antibodies against La/SSB epitopes. It was also found that immunizations with either pep or cpep led to the appearance of antibodies against the immunogen peptide by day 31 which subsequently was followed by antibody production to its complementary peptide by day 55. Using the complementary epitopes as inhibitors of the anti-idiotypic antibodies, we were able to recover the hidden anti-La/SSB reactivity in patient sera [13]. This methodology was applied in 44 anti-La (−), anti-Ro/ANA (+) sera from patients with SLE and Sjogren's syndrome. Ninety-four percent of Sjogren's syndrome sera and 80% of SLE sera were found negative for anti-pep 349–364 antibodies in ELISA prior to the treatment. After the heat+complementary epitope treatment, all SS and SLE sera became positive for anti-epitope 349–364 antibodies, while none

of the normal sera exhibited a positive reaction. Thus, virtually all anti-Ro/ANA (+) sera possess also hidden anti-La/SSB antibodies that can be unmasked by treatment with the complementary epitope [13].

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33.
B cell monoclonal proliferation in Sjogren's syndrome

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Over the past years numerous studies in humans linked several different autoimmune diseases with malignant lymphoproliferation. Adequate studies establish strong associations between B cell lymphomas and Sjogren's syndrome (SS) (1), and autoimmune thyroiditis (2). There are weaker associations between B cell lymphomas and systemic lupus erythematosus and rheumatoid arthritis.

Among all autoimmune diseases, SS (autoimmune epithelitis) (3) best illustrates the autoimmunity-lymphoproliferation-lymphoma sequence. The SS-associated lymphoproliferation ranges from an increased frequency of mixed monoclonal cryoglobulinemia, increased levels of circulating CD5-positive B cells, circulating monoclonal immunoglobulin, to an increased frequency of malignant non Hodgkin's lymphomas (NHL) (4–6). A notable histological feature in a parotid gland is the lymphoid follicle-like structures with germinal centers that simulate the architecture of peripheral lymphoid nodes where B-lymphocytes are often oligoclonal (7) with a