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Anti-Sm and anti-RNP antibodies

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Abstract

Among anti-nuclear antibodies, anti-Sm and anti-RNP antibodies are of the utmost importance in clinical practice. Anti-Sm antibodies are directed against 7 proteins (B/B′, D1, D2, D3, E, F, G) that constitute the common core of U1, U2, U4 and U5 small nuclear ribonucleoprotein (snRNP) particles; B/B′, D1 and D3 are more frequently targeted. Anti-RNP antibodies react with proteins (70 Kd, A, C) that are associated with U1 RNA and form U1snRNP. Anti-Sm and anti-RNP antibodies are directed towards both discontinuous and linear epitopes which are either contained in the protein sequence or are post-translationally modified.

The assays to detect anti-Sm and anti-RNP antibodies are counterimmunoelectrophoresis (CIE), immunoblot, and ELISA, based on purified or recombinant proteins or synthetic peptides.

Anti-Sm antibodies are detectable in a percentage of SLE patients comprised between 5 and 30%; they are more prevalent in blacks and because of their high specificity for SLE have been included in the serological criteria for diagnosing the disease.

Anti-RNP are detectable in 25–47% of SLE patients; high titers of anti-RNP antibodies are diagnostic of mixed connective tissue disorder (MCTD). The measurement of anti-Sm and anti-RNP antibodies is more important in the diagnosis of SLE than in the follow-up of patients. However, anti-RNP antibodies are more prevalent in patients with Raynaud's phenomenon and are associated with milder renal involvement. On the contrary, anti-Sm antibodies are associated with the severity and the activity of renal involvement.

The specificity of anti-Sm antibodies, together with epidemiological data, suggest that Epstein-Barr virus infection has the potential to induce anti-Sm antibodies by molecular mimicry.

Anti-nuclear antibodies, a hallmark of the systemic autoimmune diseases, include several populations of antibodies with different specificities. Among them, anti-Sm and anti-RNP antibodies are of the utmost importance in clinical practice; in research, the study of the mechanisms inducing their production has opened up new perspectives and helped to elucidate the pathogenesis of autoimmune disorders.

Keywords: *Anti-Sm, anti-RNP, autoantibodies, systemic lupus*

Sm and RNP: Their structure

Anti-Sm antibodies are directed against 7 proteins (B/B′, D1, D2, D3, E, F, G) that constitute the common core of U1, U2, U4 and U5 small nuclear ribonucleoprotein (snRNP) particles [1,2]. The anti-Sm autoimmune response is directed against the proteins B/B′, D1 and D3, and to a lesser extent D2. The E, F and G proteins are recognized less frequently and only under native conditions [3,4]. Anti-RNP antibodies react with proteins (70 Kd, A, C) that are associated with U1 RNA and form U1snRNP (Figure 1).

The snRNP are RNA-protein complexes that are abundant in the nuclei of all eukaryotic cells; together with many additional proteins they form the spliceosome, a structure involved in the nuclear processing of pre-mRNA. The biogenesis of snRNP is a multi-step process that takes place primarily in the cytoplasm, where the protein components are synthesized and the RNAs (products of RNA polymerase II) are transported.

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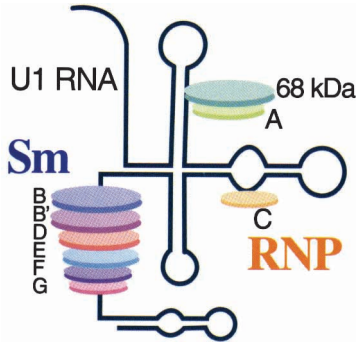

Figure 1. Structure of U_1 sn RNP. The figure illustrates the protein components of the U_1 sn RNP particle, complexed with RNA.

The 7 core proteins are stored in the cytoplasm as a pentamer (D1, D2, E, F, G) and a complex of B, D3 and D1 to which the methylosome (or PRMT5 complex) associates. Methylosome contains a type II methyltransferase which catalyzes the conversion of arginines to symmetrical dimethylarginines. This post-translational modification affects the carboxy-terminal parts of B/B′, D1 and D3 [5], and sequences of these proteins containing symmetrical dimethyl-arginines are specifically recognized by SLE autoantibodies [6].

Methylation markedly increases the affinity of Sm proteins for the SMN complex, the second mediator in snRNP assembly, which transfers Sm proteins to U snRNA [7]. In the presence of U snRNA the Sm proteins assemble in a ring into which the snRNA is inserted. Post-translational modifications in RNA and specific determinants of the proteins constitute nuclear localization signals that direct the migration of the particles to the nucleus.

The Sm proteins are highly conserved in eukaryotes; all of them (except F) are endowed with a strong positive charge and display structural homology in a region of 70–90 aminoacids that contains the Sm1 and Sm2 motifs involved in protein–protein interactions.

Anti-Sm and anti-RNP: Their frequency

Anti-Sm antibodies are detectable in a percentage of SLE patients comprised between 5 and 30%. Studies in North America generally cite a prevalence of around 30% [8], but European studies report lower frequencies of around 5% [9]. It has been suggested that ethnic differences in the populations studied may explain this difference in frequency; for example, anti-Sm antibodies are known to be more prevalent in blacks [9–11].

Anti-Sm antibodies are almost always associated with anti-RNP and in the few cases in which anti-Sm alone were initially detected, anti-RNP developed later in the course of the disease. Because of their high specificity for SLE, anti-Sm antibodies have been included in the serological criteria for diagnosing the disease [8].

Anti-RNP are detectable in 25–47% of SLE patients [12,13]. High titers of anti-RNP antibodies are diagnostic of MCTD.

Anti-Sm antibodies are also present, in a frequency similar to that observed in the human disease, in the MRL-lpr/lpr mouse, a strain that spontaneously develops SLE [14].

Anti-Sm and anti-RNP: Their detection

Anti-Sm and anti-RNP antibodies can be detected by different type of assays: counterimmunoelectrophoresis (CIE), immunoblot, and ELISA.

CIE was the very first method proposed for the measurement of these autoantibodies [15] and is still widely used today. The source of the antigens is usually rabbit thymus extract. It is generally agreed that this method is characterized by a low sensitivity counterbalanced by a high specificity [16,17]. A modification of the method was recently proposed [18]: by allowing sera diffusion before electrophoresis the sensitivity of the assay can be slightly improved. This method requires technical expertise and is time-consuming, but the cost of typing autoantibody specificities by CIE is 10–30 times lower than by ELISA [19].

The immunoblot was originally performed using cell lysates, generally from HeLa cells or rabbit thymus lysates [20]. It is more sensitive than CIE [21], but is technically quite complex to perform and the identification of antigens is difficult because of the high number of bands usually obtained with SLE sera.

Anti-RNP autoantibodies are detected more frequently by CIE than by immunoblot [22], whereas anti-Sm detection by CIE is more difficult [22,23].

ELISA assays are widely used in the form of commercially available kits and are in general highly sensitive. They are easy to perform, do not require either complex instrumentation or specific training, can easily be automated, and the results are obtainable within a few hours. These assays are potentially both accurate and reliable, but the currently available products vary greatly in terms of sensitivity [24] and specificity [21]. Nevertheless, most assays are able to measure the anti-Sm antibody titer with reasonable accuracy [25].

Recently, ELISA kits produced by twelve different manufacturers were compared with CIE and IB using eleven serum samples from patients with systemic autoimmune diseases. Overall the detection of Sm, regardless of the method, was 47% while the detection of RNP was 100% [26].

Better agreement in the detection of anti-RNP than anti-Sm has been reported by other authors, who compared either CIE, ELISA and two immunoblotting assays [27] or CIE, ELISA and LIA [19].

The conflicting results obtained with different ELISA assays as far as anti-Sm detection is concerned can be at least partially explained by the different antigens used on the solid phase. In the coating either extractive or recombinant Sm proteins may be used, or else synthetic peptides that may either be unmodified or contain modified aminoacids.

Discrepancies between the results of immunoblot vs ELISA or CIE are often observed; possible explanations are the different sources of antigens and the loss of conformational determinants attributable to the different procedures used in the gel separation of the antigens [28,29]. Moreover, it should be stressed that recombinant proteins do not always contain all the epitopes expressed on the "native" antigen; in recombinant proteins post-translational modifications may be absent or "incorrect" or additional sequences that affect antibody recognition may be present.

Recently line immunoassays based on recombinant proteins have been employed for anti-Sm and anti-RNP detection, and have demonstrated high specificity and high sensitivity [30–32]. Both line immunoassays and CIE were shown to be better correlated than ELISA with the clinical diagnosis [19].

Anti-Sm and anti-RNP: Their clinical significance

The clinical significance of anti-Sm and anti-RNP antibodies is still a matter of debate. Serial determinations of these antibodies have shown variations in titer over time, raising the possibility that they may be linked to exacerbations and remissions of the disease. Therefore, a number of studies have tested their possible associations with single disease manifestations, with the severity of organ involvement, or with disease activity [33].

Single disease manifestations

Anti-RNP antibodies have repeatedly been found to be associated with Raynaud's phenomenon [34,35]. In contrast, the association between anti-Sm antibodies and CNS injury remains uncertain [33,36,37].

In 1978 Winfield et al. [36] reported the results of a study of 25 SLE patients who were followed during 29 acute episodes of central nervous system (CNS) disease. They found an increased incidence of anti-Sm antibodies in patients with CNS dysfunction. Anti-Sm antibodies were in fact observed in 71% of the patients with isolated CNS disease and only in 25% of unselected patients.

Discordant results have also been obtained in the analysis of the prognostic value of anti-Sm antibodies. Winn reported a higher prevalence of anti-dsDNA than anti-Sm antibodies ($p < 0.005$) in patients with serious CNS disease and suggested that anti-Sm may identify a subset of SLE patients with milder renal disease and CNS involvement [37]. Janwityanuchit on the contrary found that patients with anti-Sm antibodies suffer more frequently from neuropsychiatric manifestations than patients with anti-dsDNA alone ($p < 0.05$) [33].

Severity of organ involvement

Anti-Sm and anti-RNP antibodies seem to exert opposite effects on the severity of renal involvement. According to some authors, anti-RNP antibodies are associated with milder renal involvement [12,33,38]. One exception is the prospective study by H.M. Bastian [39], which found an association between lupus nephritis and anti-RNP antibodies. The different ethnic backgrounds of the patients included in this study could explain this discrepancy.

The presence of anti-Sm antibodies has been reported to be related to renal disease and this association is even stronger when anti-Sm are found together with anti-dsDNA [33]. In a recent study by Alba et al. [38], a higher frequency of anti-dsDNA, anti-RNP, anti-Sm and lupus anticoagulant was found in lupus nephritis patients (127 patients with biopsy-proven nephritis) compared with the control group (206 randomly selected SLE patients without nephritis).

Disease activity

Barada et al. [40] reported that rising titers of anti-Sm correlated with a clinical exacerbation of CNS and non-CNS disease in 60% of their patients and that anti-Sm predicted a disease flare in 50%. A correlation between anti-Sm antibodies and disease activity was also noted in a Finnish study conducted on 17 SLE patients [41], and in a later study performed on 51 consecutive SLE patients selected according to ARA criteria [42]. All 8 patients with both anti-Sm and anti-dsDNA were found to have active SLE, and pulmonary, renal and CNS involvement were the most prominent manifestations of their disease activity. On the basis of these findings, the authors suggest that the activity of the disease is related to the production of multiple autoantibody specificities [41,43].

Anti-Sm and anti-RNP: Their specificity

Anti-Sm and anti-RNP antibodies from SLE patients are directed towards both discontinuous and linear epitopes which are either contained in the protein sequence or are post-translationally modified.

cDNA isolates encoding Sm B and B′ and cloned from different tissues have been found to be identical except for one or two aminoacids [44–46]. Rokeach et al. [47] analyzed the reactivity of SLE sera with truncated recombinant proteins spanning the entire length of the B/B′ protein and identified a dominant

epitope characterized by a series of proline and glycine repeats in the carboxyl terminus of the protein. Conformational epitopes were also detected in the N-terminal portion of the molecule [47]. Human and mouse lupus sera reacted similarly with these proline-rich sequences [48]. A more detailed mapping of the linear epitopes was performed using synthetic peptides [49] and led to the identification of a sequence characterized by the motif PPPG(I,M)(R,K) as the target of the autoantibodies.

SmD1, a polypeptide of 119 aminoacids, was cloned in 1988 by Rokeach et al. [50]. Because of the relatively small size of this molecule, analysis of the antigenic regions was conducted using only synthetic peptides. By overlapping synthetic peptides, several immunoreactive regions were in fact identified on SmD1, and SLE sera reacted with the N-terminal [51], the central [51] and the C-terminal sequences [51,52]. The presence of a dominant epitope in the C-terminal region of the molecule was subsequently confirmed by other studies [53–55]. As described for the SmB epitope, MRL-lpr/lpr sera reacted with the same epitope that was recognized by human SLE sera [53].

The synthetic peptides used as the dominant epitope in the above cited studies differ in length (Table I), but all include the stretch consisting of 9 gly-arg, aa 97–114. Even if the ELISA assays employing these peptides on the solid phase are not identical, the frequency of positive SLE sera detected using different assays is similar, being comprised (with only one exception [54]) between 25 and 36%. In most cases, sera reactive with the C-terminal synthetic peptides decorate SmD1 in immunoblot [52,55].

Epitopes recognized by SLE sera have also been defined on SmD2 and SmD3, and all reside in charged sequences that are accessible on the surface of the proteins, even when they are complexed in the spliceosome [56]. One of the SmD3 epitopes has a high sequence homology with the C-terminal region of SmD1 [56]. Thus, antibodies specific for one of the Sm proteins also crossreact with other Sm components. This crossreaction can be due to the sharing of a sequence, as in the case of SmD1 and D3, or to conformational epitopes of one protein mimicking the linear epitopes of another.

Sm B/B′, D1 and D3 are post-translationally modified in the arginines, which are methylated in the aminic residues of their side chains [5]. Other nuclear proteins containing gly-arg repeats are asymmetrically dimethylated, but Sm and myelin basic protein are the only known human proteins that bear symmetrically dimethylated arginines [6]. The gly-arg repeats 97–114 of SmD1, synthesized with dimethylated arginines, were specifically recognized by most SLE sera containing anti-Sm antibodies [6].

Anti-Sm antibodies are frequently of the IgG isotype, which is typical of a T-cell dependent response. Such a response is directed to a limited number of epitopes of SmB and D, that do not overlap the B epitopes and that correspond to the Sm motifs, sequences which are conserved among the Sm proteins and are important for protein–protein interactions [57]. In addition, however, the C-terminal SmD1 sequence contains T epitopes; T cells specific for this sequence have in fact been isolated from immunized NZB × NZW mice [58].

Sera containing anti-RNP antibodies react with epitopes located in the proteins 70 Kd, A and C. The U1-70 Kd protein has multiple antigenic regions that have been identified by means of truncated recombinant proteins [59–61], and contain both linear and conformational epitopes [61].

SLE and MCTD sera differ in the frequency with which the different epitopes are recognized. A small number of MCTD sera do not react with the proteins alone but specifically recognize the complex of UI RNA and 70 kd or A protein [62]. The U1-70 Kd protein is cleaved by caspases in UV-irradiated keratinocytes [63], but is also susceptible to the metal-catalyzed oxidative cleavage that takes place during ischemia-reperfusion [64]. Apoptotically modified forms of U1-70 kd are preferentially recognized by SLE patients with skin involvement, while the oxidized form is more frequently bound by patients with Raynaud's phenomenon [65].

U1-A contains 2 major epitopes, both located on the surface of the molecule; only one of them, however, is exposed in the U1snRNP particle and, when used as immunogen in rabbits, can induce a response against multiple snRNP proteins [66].

T cell clones specific for U1-70 kd have been isolated from SLE and MCTD patients [67–69], most of whom display the Th0 phenotype [69].

In animal models, a phosphorylated peptide corresponding to 131–151 of the U1-70 kd protein

Table I.

Sequences of SMD-derived peptides	Length	Reference
$_{97}$GRGR GRGRGRGRGR GRGRGGPRR$_{119}$	23 aa	Barakat et al. (51)
$_{95}$VAGRGR GRGRGRGRGR GRGRGGPRR$_{119}$	25 aa	Sabbatini et al. (52)
$_{95}$VAGRGR GRGRGRGRGR GRGRGGPRR$_{119}$	25 aa	James JA et al (53)
$_{83}$VEPKVKSK KREAVAGRGR GRGRGRGRGR GRGRGGPRR$_{119}$	37 aa	Riemekasten et al. (54)
$_{83}$VEPKVKSK KREAVAGRGR GRGRGRGRGR GRGRGGPRR$_{119}$	37 aa	Jaekel HP et al. (55)

was found to be a T-cell epitope in MRL-lpr/lpr mice; moreover, intravenous administration of this peptide downregulated anti-DNA antibody production and prolonged the survival of the treated animals [70].

Anti-Sm and anti-RNP: Mechanisms of induction

The mechanisms that lead to anti-Sm antibody production have formed the object of thorough investigation. Interesting clues have emerged from the analysis of the antigenic regions of Sm.

Epitope mapping of SmD revealed the existence of a dominant epitope in the C-terminal region of the molecule that bears a striking homology to a sequence of the viral protein EBNA I [71]. The sequence homology encompasses a gly-arg stretch of 11 consecutive aminoacids, with 4 other identical aminoacids and 4 conservative substitutions over a total length of 24 aminoacids. Such an extensive homology is perceived by the immune system and leads to the production of crossreactive antibodies. In fact, antibodies specific for SmD 95–119 react with the homologous EBNA I sequence 35–58 and recognize it in the context of the whole EBNA I molecule. Conversely, immunization with the EBNA I peptide induces antibodies reactive with SmD [71].

EBNA I is a protein encoded by the EBV genome and expressed in the nuclei of infected cells in the early phases of infection. The EBNA I gene ensures replication of the viral genome during cell division. In latent infection it is transcribed both in the growth programme, when the virus expresses all the EBNA and LMP proteins, and in the default programme, when the virus expresses only EBNA I and LMPs, and it persists in infected memory B cells [72].

The EBNA I protein has an unusual structure in that it contains in its central part a gly-ala repeat which constitutes one-third of the molecule [73] and represents a dominant epitope in the anti-EBNA I immune response that follows EBV infection [74]. However, antibodies against other portions of the molecule are present in immune sera. Normal subjects, as well as those affected by autoimmune or EBV-related disorders (nasopharyngeal carcinoma, Burkitt's lymphoma), produce antibodies to the N-terminal EBNA I sequence 35–58, but only in SLE patients these antibodies are crossreactive with SmD1 [75].

Another sequence of EBNA I, PPPGRRP- aa398-404, is strongly homologous to the dominant epitope of SmB. Immunization with either the viral or the autoantigen sequence induces lupus-like autoimmunity in animals [76].

Taken together, these observations suggest that EBV infection has the potential to induce anti-Sm antibodies by molecular mimicry. Epidemiological data strongly support this view, suggesting that SLE is linked to previous EBV exposure. Because of the high prevalence of EBV infection in adults, in a recent study seroconversion against EBV was tested in children and young adults with SLE and compared to age-matched controls. Virtually all the SLE patients had anti-viral antibodies compared to 70% of the controls [77]. In adults with SLE, the difference was less marked (99.5 vs 94.4%), but still significant [78].

A role of molecular mimicry in the induction of anti-RNP antibodies as well has been suggested. In fact, a subset of anti-U1-70 kd antibodies reacts with a sequence of 5 aminoacids that is also contained in the influenza B matrix protein [79], and the retroviral p30gag antigen is homologous to an antigenic portion of U1-70 kd [80].

Take Home Messages

- The measurement of anti-Sm and anti-RNP antibodies is more important in the diagnosis of SLE than in the follow-up of patients.
- Despite the recent development of more sensitive assays, the detection of anti-Sm antibodies remains difficult.
- At least two different assays should be used for the detection of anti-Sm antibodies.
- Some of the T and B epitopes on Sm and RNP proteins are post-translationally modified.
- Molecular mimicry plays a role in the induction of anti-Sm and anti-RNP antibodies.

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