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Anti-Sm and Anti-U1-RNP Antibodies: An Update

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Abstract

From their discovery anti-Sm autoantibodies (Ab) have been associated with systemic lupus erythematosus (SLE), while anti-U1-RNP Ab detected alone are predominant in patients with mixed connective disease (MCTD). However, the identification of anti-Sm/U1-RNP Ab in a patient may be challenging, and usually requiring a two-step process including a screening step performed by indirect immunofluorescence (IIF) on HEp-2 cells showing a coarse speckled nuclear staining at an elevated level, followed by a confirmatory assay using specific antigens. The recent development of novel assays and the characterization of the target epitopes have been beneficial to improve the sensitivity for anti-Sm/U1-RNP Ab detection, but, in some cases, the necessity to use a different assay remains mandatory. Another recent and unexpected observation is related to the suspected role played by environmental and epigenetic factors in the induction of anti-Sm/U1-RNP Abs. Altogether, better knowledge regarding anti-Sm/U1-RNP Ab will undoubtedly provide improvements for the management and treatment of these patients.

Keywords: Systemic lupus erythematosus; Mixed connective disease; Autoantibodies; Anti-Sm; Anti-U1-RNP

Historical Points

Tan et al. in 1966 first identified a novel extractable anti-nuclear autoantibody (Ab) from a patient with systemic lupus erythematosus (SLE), Mrs. Stephanie Smith, and now the Ab is referred to as anti-Sm Ab [1]. Five years later, another extractable nuclear antigen (ENA) autoAb, initially named anti-Mo Ab, which co-exists with anti-Sm Ab and is sensitive to the action of RNase, was reported [2]. The anti-Mo Ab is actually defined as anti-nRNP, anti-RNP or better, anti-U1-RNP Ab. Next, the structure of the complex known as the spliceosome, which is composed of U-rich small nuclear ribonucleic acids (snRNAs) associated with Sm and other snRNP antigens, was determined explaining, in turn, why anti-Sm/U1-RNP Abs are frequently associated [3]. This breakthrough was accompanied by the emerging concept that anti-Sm Ab are highly specific for SLE, whereas elevated anti-U1-RNP Ab are predominant in patients with mixed connective disease (MCTD) which refers to patients with overlapping features of SLE, systemic sclerosis (SSc), and autoimmune myopathy with low steroid requirement and a good prognosis [4]. Since that time the term MCTD, as a distinct disease, is controversial due to the evolution of a subgroup of patients with another connective tissue disease [5] and, therefore, some authors prefer the term undifferentiated autoimmune rheumatic disease [6].

Autoantigen

The Sm core protein is composed of an heptamer (B or B′, D1/D2/D3, E, F, and G ranging from 13 to 28 kDa), organized in a ring-like structure into which the snRNA is inserted, and that can interact with each of the U-rich snRNAs to form U1, U2, U4-6, and U5 snRNPs (Figure 1). The Sm B and Sm B' proteins are two alternative products from a single gene, and each snRNP includes specific proteins: U1-70 kDa for U1-snRNPs, U2-A' and U2-B for U2-snRNPs, while U5-200 kDa with other specific proteins define U4/U6 and U5 snRNPs.

Anti-snRNPs are classified into four groups: first, anti-Sm Ab that recognize B/B' and D1/D2/D3 shared by the U1, U2, U4/6 and U5 snRNPs; second, anti-U1-RNP Ab that recognize the U1 plus the A-34 kDa and C-23 kDa partners; third, a minor and rare group of Ab reported only in a few cases that recognize U2-RNP, U4/U6-RNP, U5-RNP or the 5'-trimethylguanosine cap structure that recognize U1-U5-RNAs; and fourth, for some patients the anti-Sm/U1-RNP Abs react with a cross-reactive epitope PPPG(I,M)(R,K) and/or the quaternary structure of the spliceosome that contains the Sm core particle, U1-C and U1-A particles, thus defining an over-lapping group [7]. This recognition is explained by the fact that the assembly of the spliceosome is sequential during the binding of the complex to the splicing sites present in the introns of the pre-mRNA, leading to intron excision and ligation of the exons to form the mature messenger RNA [8].

Anti-snRNPs are directed towards both linear and continuous epitopes which are either present on the native protein or after post-translational modifications, such as modifications important for controlling the translocation from the cytoplasm to the nucleus. Conversion of the arginines present in the carboxyterminal parts of B, D1 and D3 into symmetrical di-methylarginines by type II methyltransferases, was described to be specifically restricted to SLE patients [9]. By contrast the E, F and G proteins of the Sm heptamer are not methylated on their arginines, thus explaining that Ab recognition is less frequent and restricted to the native forms. For the U1 protein, the oxidized form has been associated with patients with Raynaud's phenomenon, while the apoptosis-related modified form was associated with SLE patients presenting skin involvement [10].

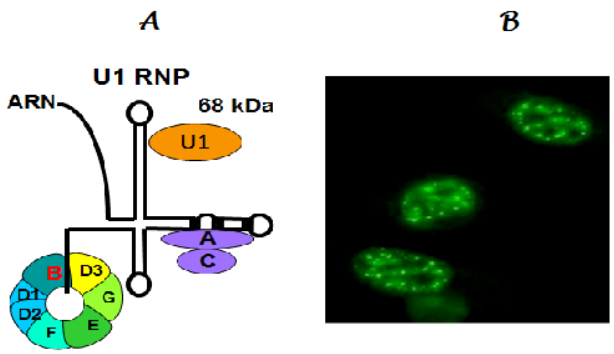

Figure 1: Anti-Sm/U1-RNP antibodies recognize ribonucleoproteins (RNP). **A**- four kinds of polypeptides bound to U1-RNA: U1-RNP, protein A, protein C, and the Sm heptamer (B or B′, D1/D2/D3, E, F, and G) organized in a ring-like structure into which the U1-RNA is inserted. **B**- Anti-Sm/U1-RNP Ab screening by indirect immunofluorescence on HEp-2 cells reveals a coarse speckled nuclear pattern without nucleolar and cytoplasmic staining.

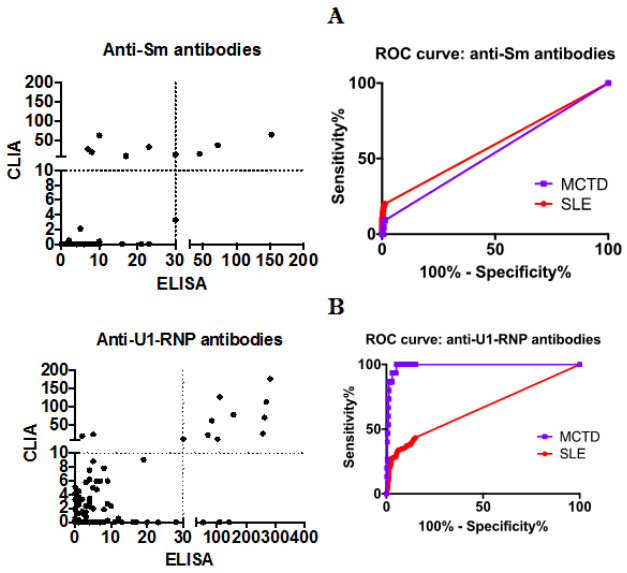

Figure 2: Anti-Sm and anti-U1-RNP Abs. **A**- Comparison of solid phase chemiluminescence immunoassay (CLIA, IDS-Isys, France) and enzyme-linked immunosorbent assay (ELISA, Immuno concept, USA) for anti-Sm/U1-RNP antibody detection. **B**- Receiver operating characteristic (ROC) analysis of anti-Sm and anti-U1-RNP CLIA (IDS-Isys) autoantibodies (Ab) revealing that anti-Sm Ab are associated with systemic lupus erythematosus (SLE), while anti-U1-RNP Ab detected alone are predominant in patients with mixed connective disease (MCTD).

Detection

The identification of anti-Sm Ab and anti-U1-RNP Ab is usually a two-step process with initially a screening step typically performed using indirect immunofluorescence (IIF) staining of the human larynx carcinoma cell line HEp-2 revealing a coarse speckled nuclear staining (Figure 1). This represents the distribution of the snRNP particles in the non-chromosomal regions of the nucleus, or nucleoplasm. Second, a confirmatory assay is performed using specific antigens. Although immune precipitation (IP) using S35-methionine labeled cell extract is considered as the gold standard, the more widely used assay technologies are based on enzyme-linked immunosorbent assays (ELISA), addressable laser bead immunoassays (ALBIA), line immunoassays (LIA), and, more recently, multiplexed immunoassays including chemiluminescent immunoassay (CLIA). Along with slight differences observed between the different technologies (Figure 2), another source of discrepancies is related to the use of synthetic peptides, recombinant antigens or affinity purified antigens from calf thymus, rabbit thymus, or human cell lines.

Several points have to be considered when testing anti-Sm and anti-U1-RNP Ab in clinical practice: (1) IFI titers are usually very high on HEp-2 cells (≥ 1:1280); (2) due to their unique inter-relationship, virtually all patients with anti-Sm Ab react both with Sm and U1-RNP; (3) in some cases, anti-U1-RNP Ab developed after anti-Sm Ab in the course of the disease, however, a false positive result has occurred when anti-Sm Ab are detected at lower levels and when anti-U1-RNP Ab is negative; (4) in a cohort, the prevalence of the anti-U1-RNP Ab should be higher than that of anti-Sm Ab; and (5) non-SLE patients with elevated levels of anti-U1-RNP Ab tend to have more features typical of MCTD (Tables 1 and 2).

	Sm	**snRNP**
Sensitive to RNase	No	Yes
Main target	B/B' plus D1 and D3	U1-70 kDa plus A and C
Rare targets	E, F and G	U2, U4/U6 and U5
		5'-trimethylguanosine
Cross reactive epitope	**PPPG(I,M)(R,K)**	
Post-translational modifications	Arginine di-methylation	Oxidation Phosphorylation

Table 1: Characteristics of Sm and anti-small nuclear(sn) ribonucleoproteins (RNP).

	SLE	MCTD
Anti-Sm	5-22%	<5%
Anti-U1-RNP	20-50% (mild level)	~100% (high level)
Anti-A	25%	70%
Anti-C	<5%	20%

Table 2: Serological differences between patients with systemic lupus erythematosus (SLE) and mixed connective diseases (MCTD) for anti-Sm/U1-RNP antibodies.

Clinical Associations

Anti-Sm Abs are one of the serologic biomarker specified in the criteria for SLE as depicted by the American College of Rheumatology (ACR) in 1982 and confirmed in the 2012 SLICC revised classification criteria [11,12]. The higher specificity of anti-Sm Ab for SLE is counterbalanced by a lower sensitivity, as they are present in 5 to 30% of SLE patients with important ethnic differences as they are more prevalent in black (30%) than Caucasians (5%). Longitudinal follow-up studies have further observed that anti-Sm and anti-U1-RNP Abs can be detected one year and six months before the clinical manifestations of the full disease, respectively [13]. Some reports, but not all, have associated anti-Sm Ab detection in SLE with disease activity, kidney and/or central nervous involvement, and a decreased rate of complete response to treatment [14-16].

By definition all patients with MCTD are positive for anti-U1-RNP Abs at elevated titer, however they are not specific for MCTD as anti-U1-RNP Ab prevalence is found in 20-50% of SLE patients and anti-U1-RNP Ab detection is not a rare event among clinical features such as in undifferentiated connective diseases (UCTD, 6-20%), SSc, and Sjögren's syndrome. The main clinical features of anti-U1-RNP Abs are Raynaud's phenomenon, followed by swollen digits, esophageal dysmotility, leukopenia, arthritis/arthralgia, myositis, serositis, and a favorable response to steroid treatment. In addition, anti-U1-RNP Ab seems to have a protective effect on the renal involvement, and a normal complement level during lupus nephritis in patients with anti-U1-RNP Ab positivity may explain this paradox as opposed to patients with anti-Sm Ab that have complement consumption [17,18].

Last but not least, anti-A and -C protein Abs are found in up to 25% of unselected SLE patients, and the prevalence rises to 75% when considering SLE patients with anti-U1-RNP Abs. Anti-A and -C protein Abs are not correlated with disease activity and their detection varies during the course of the disease. As a consequence, anti-A and -C Ab detection is not recommended and their detection is restricted to a limited number of laboratories.

Other Associations

Anti-Sm and anti-U1-RNP Abs can be associated with hypergammaglobulinemia, and such association may be due to anti-Sm/U1-RNP Abs themselves since they can represent over 20% of the total IgG in some cases [19]. A critical role for HLA-DR3, an important risk-factor for SLE, has been further established in the lupus-prone mouse model NZM2328 for the development of an anti-Sm immune response [20].

With time, the titers of anti-Sm and/or anti-U1-RNP Abs do not fluctuate substantially in SLE, while in MCTD some authors have reported fluctuations over time. In those SLE patients treated with the B-cell depleting monoclonal antibody rituximab that recognizes CD19 on B cells and short-lived plasmablasts, but not long-lived plasmablasts, the levels of anti-Sm and anti-U1-RNP Abs are not affected, in contrast to anti-dsDNA Abs [21].

As reported for anti-SSA/SSB Abs, anti-Sm/U1-RNP Abs detection is associated with an elevated level of interferon (IFN) type I inducible genes. One explanation is that anti- Sm/U1-RNP Ab complexes can stimulate IFN type I in plasmacytoid dentritic cells through a pathway that involves Fc receptors and the endosomal Toll-like receptor (TLR)7 [22-24]. Another explanation is that IFN type I overexpression might be important for anti-Sm Ab development as observed in a patient with chronic hepatitis C treated with pegylated interferon-alpha and ribavirin [25]. Recent data have further highlighted the fact that IFN type I activation in systemic autoimmune diseases is associated with an abnormal DNA methylation process [26], leading in turn to abnormal expression of normally repressed autoantigens. Among autoantigens controlled by DNA methylation we have already highlighted SSB, KRT19, HERV-CD5 and HRES-1, and a cross-reactivity of anti-p38 Gag HRES-1 Abs with U1-RNP has also been reported [27-30].

Molecular mimicry with exogenous and endogenous viral sequences has been described for Sm/U1-RNP peptides including anti-SmD 95-119 peptide Abs that react with the Epstein Barr Virus EBNA I sequence 35-58. Immunization with the EBNA I peptide induces Abs that cross-react with the autoantigen SmD [31]. Similarly, anti-U1-RNP sequences have been shown to cross-react with sequences found in the influenza B matrix protein, the p30gag retroviral antigen, or fungal proteins [32-34].

Conclusion

Despite the dichotomy observed between anti-Sm/U1-RNP Abs for SLE, and anti-U1-RNP Ab alone for MCTD, the detection of Sm Abs remains difficult in clinical practice thus explaining that initial assays should be repeated or confirmed with a different assay. Recent advances suggest important contributions from environmental factors (viruses, UV lights) together with epigenetic factors for the emergence of anti-Sm/U1-RNP Abs. Thus, better comprehension of this pathway will have important diagnostic and therapeutic applications.

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