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

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

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.

Lemerle, Lupus Open Access 2016, 1:3

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.

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.

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

| Sm          | snRNP                  |
|-------------|------------------------|
| Sensitive to RNase | No                     |
| Main target  | B/B’ plus D1 and D3    |
| Rare targets | E, F and G             |
| Cross reactive epitope | PPPG[1,M](R,K)  |
| Post-translational modifications | Arginine di-methylation |

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).
**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/arthritisalgia, 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 dendritic 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 1 sequence 35-58. Immunization with the EBNA 1 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.

**Acknowledgements**

Many thanks to Simone Forest and Geneviève Michel, for their help with the writing of this manuscript, and to the editorial assistance of Doctor Wesley H. Brooks, University of South Florida, USA, is greatly appreciated.

**References**

1. Tan EM, Kunkel HG (1966) Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol 96: 1297-1304.
2. Mattioli M, Reichlin M (1971) Characterization of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera. J Immunol 107: 1281-1290.
3. Renaudineau Y, Hillion S, Saraux A, Youninou P (2014) Traité des maladies et syndromes systémiques. Flammarion Médecine Sciences 1-32.
4. Sharp GC, Irvin WS, LaRoque RL, Velez C, Daly V, et al. (1971) 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 1 sequence 35-58. Immunization with the EBNA 1 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].

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

|           | 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%      |

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5. Cappelli S, Bellando Randone S, Martonvic D, Tamas MM, Pasalic K, et al. (2012) "To be or not to be," ten years after: evidence for mixed connective tissue disease as a distinct entity. Semin Arthritis Rheum 41: 589-598.

6. Ciang NC, Pereira N, Isenberg DA (2016) Mixed connective tissue disease-enigma variations? Rheumatology (Oxford) (In press).

7. James IA, Harley JB (1992) Linear epitope mapping of an Sm B/B' polypeptide. J Immunol 148: 2074-2079.

8. Stark H, Dube P, Lührmann R, Kastner B (2001) Arrangement of RNA and proteins in the spliceosomal U1 small nuclear ribonucleoprotein particle. Nature 409: 539-542.

9. Chang HH, Hu HH, Lee YJ, Wei HM, Fan-June MC et al. (2013) 9.

10. Maddison PJ, Reichlin M (1977) Quantitation of precipitating antibodies to certain soluble nuclear antigens in SLE. Arthritis Rheum 20: 819-824.

11. Chowdhary VR, Dai C, Tilahun AX, Hanson JA, Smart MK, et al. (2015) A Central Role for HLA-DR3 in Anti-Smith Antibody Responses and Glomerulonephritis in a Transgenic Mouse Model of Spontaneous Lupus. J Immunol 195: 4660-4667.

12. Seret G, Hanrotel C, Bendaoud B, Le Meur Y, Renaudineau Y (2013) Homozygous FCGR3A-158F mutation is associated with delayed B-cell depletion following rituximab but with preserved efficacy in a patient with refractory lupus nephritis. Clin Kidney J 6: 74-76.

13. Balboni I, Niewold TB, Morgan G, Limb C, Eloranta ML, et al. (2013) Interferon-α induction and detection of anti-ribo, anti-la, anti-sm, and anti-rnp autoantibodies by autoantigen microarray analysis in juvenile dermatomyositis. Arthritis Rheum 65: 2424-2429.

14. Halloum R, Franek BS, Kariuki SN, Rhee L, Mikolaitis RA, et al. (2010) Genetic variation at the IRF7/PHRF1 locus is associated with autoantibody profile and serum interferon-alpha activity in lupus patients. Arthritis Rheum 62: 553-561.

15. Onishi S, Nagashima T, Kimura H, Matsuyama Y, Yoshio T, et al. (2010) Systemic lupus erythematosus and Sjögren’s syndrome induced in a case by interferon-alpha used for the treatment of hepatitis C. Lupus 19: 753-755.

16. Renaudineau Y, Ballestar E. (2016) Epigenetics: DNA methylation signatures in Sjögren syndrome. Nat Rev Rheumatol 12: 565-566.

17. Konsta OD, Charras A, Le Dantec C, Kapsogeorgou E, Bordron A, et al. (2016) Epigenetic modifications in salivary glands from patients with Sjögren’s syndrome affects cytokeratin 19 expression. Bull Group Int Rech Sci Todant Omotol 53: e01.

18. Konsta OD, Le Dantec C, Charras A, Corne C, Kapsogeorgou EK, et al. (2016) Defective DNA methylation in salivary gland epithelial acini from patients with Sjögren’s syndrome is associated with SSB gene expression, anti-SSB/ LA detection, and lymphocyte infiltration. J Autoimmun 68: 30-38.

19. Fali T, Le Dantec C, Thabet Y, Jousse S, Hanrotel C, et al. (2014) DNA methylation modulates HRE5I/p28 expression in B cells from patients with Lupus. Autoimmunity 47: 265-271.

20. Garaud S, Le Dantec C, Jousse-Joulin S, Hanrotel-Sallou C, Saraua, et al. (2009) IL-6 modulates CDS expression in B cells from patients with lupus by regulating DNA methylation. J Immunol 182: 5623-5632.

21. Sundar K, Jacques S, Gottlieb P, Villars R, Benito ME, et al. (2004) Expression of the Epstein-Barr virus nuclear antigen-1 (EBNA-1) in the mouse can elicit the production of anti-dsDNA and anti-Sm antibodies. J Autoimmun 23: 127-140.

22. Goldner HH, Netter HJ, Szoesteck I, Jagger E, Will H (1990) Human anti-p68 autoantibodies recognize a common epitope of U1 RNA containing small nuclear ribonucleoprotein and influenza B virus. J Exp Med 171: 819-829.

23. Query CC, Keene JD (1987) A human autoinmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30ag antigen. Cell 51: 211-220.

24. Guarreri F, Guarreri B, Borgia F, Guarrerri C (2011) Potential role of molecular mimicry between human U1-70 kDa and fungal proteins in the development of T-cell mediated anti-U1-70 kDa autoimmunity. Immunopharmacol Immuno toxicol 33: 620-625.