

Challenges and Controversies in Autoantibodies Associated with Systemic Rheumatic Diseases

Michael Mahler^{*,1}, Reinout Raijmakers² and Marvin J. Fritzler³

¹*Dr. Fooke Laboratorien GmbH, Neuss, Germany*

²*Department of Biomolecular Chemistry, Radboud University Nijmegen, Nijmegen, The Netherlands*

³*Faculty of Medicine, University of Calgary, Calgary, Canada*

Abstract: Since the first identification of self-reactive antibodies in systemic lupus erythematosus and other systemic autoimmune rheumatic diseases, many autoantibodies have been identified as useful probes in molecular and cell biology and as diagnostic and prognostic biomarkers in clinical immunology. Among the autoantigens, double-stranded desoxyribonucleic acid (dsDNA), the Smith antigen (Sm), ribonucleoproteins (RNP), Scl-70 (topoisomerase I), proliferating cell nuclear antigen (PCNA), and others were described as serologic hallmarks in the diagnosis of systemic autoimmune rheumatic diseases. Despite these advances in identifying autoantibody markers, a number of challenges and controversies persist concerning their origin, clinical usefulness and relevance. These include the association between anti-ribosomal P antibodies and clinical features in systemic lupus erythematosus, the disease specificity of several autoantibodies (i.e. chromatin, Jo-1, alpha-fodrin, topo I and CENP), the relationship between the SS-A/Ro52 and SS-A/Ro60 autoantibody system(s) and the detection of anti-RNP/Sm and anti-fibrillarin antibodies.

Keywords: Autoantibody, SLE, dsDNA, systemic rheumatic disease.

INTRODUCTION

Since the first identification of self-reactive antibodies in systemic lupus erythematosus (SLE) and other systemic autoimmune rheumatic diseases (SARD), many scientists and clinicians have contributed to a broad knowledge of autoantibodies and their usefulness as probes in molecular and cell biology, and as diagnostic and prognostic biomarkers in clinical immunology [1-5] (reviewed in [6]). The pioneers in this field were able to identify several autoantigens including double-stranded desoxyribonucleic acid (dsDNA), the small nuclear ribonucleoproteins (snRNP) known as the Smith antigen (Sm), Scl-70 (topoisomerase I; topo-I), Sjögren Syndrome (SS-) antigen A (SS-A, Ro 52 kDa and Ro 60 kDa) and SS-B (La 48 kDa), U1-RNP, ribosomal phosphoproteins (Rib-P), and centromere proteins (CENPs) as hallmarks in the diagnosis of SARD [1-5, 7] (reviewed in [6, 8]). The name of an autoantibody system was at the discretion of the investigators first describing it and this included choices based upon subcellular localization or function (e.g. CENP), the name of the index patient providing the prototype serum (Sm, Ro, La, Jo-1, Sa), the disease of the first patients studied (SS-A, SS-B, Scl-70), or a distinctive molecular feature of the autoantigen (e.g. RNP, GW) (reviewed in [6, 9]). Human autoantibodies have been used as biological tools to study cellular processes in which the cognate autoantigens are involved. By this approach, anti-Sm antibodies have been used to characterize and elucidate the splicing and processing of heterogeneous nuclear ribonucleic acid (hnR NA), a precursor to messenger RNA [10]. Moreover, autoantibodies have been utilized to establish the diagnosis of patients suffering from SARD such as SLE, systemic scler-

osis (SSc), mixed connective tissue disease (MCTD), primary Sjögren Syndrome (SjS), dermatomyositis (DM) and rheumatoid arthritis (RA) (reviewed in [6]). Numerous research studies and reviews have been published describing the biological function of autoantigens, the sero-genetic associations and the disease mechanisms of autoantibodies. Despite all these advances in autoantibody detection, a number of controversies persist.

Therefore, the present review is focussed on some key challenges and controversies that the application of autoantibodies to the diagnosis and management of SARD has engendered. These include the association between anti-Rib-P antibodies and clinical features in SLE, the disease specificity of certain autoantibodies such as chromatin, Jo-1, alpha-fodrin, topo-I and CENP, the relationship between the SS-A/Ro52 and SS-A/Ro60 autoantibody system(s) and the detection of anti-RNP/Sm and anti-fibrillarin antibodies (AFA).

DISEASE SPECIFICITY OF AUTOANTIBODIES

When laboratories collect samples to establish the clinical relevance of autoantibodies, they often collect samples based on immunological reactivity with the respective antigen and retrospectively assign the diagnosis of the patient. Since the frequency and prevalence of the various autoimmune diseases is significantly different and the frequency of various autoantibodies depends on the focus of the investigating laboratory, it can be difficult to arrive at a consensus about the clinical associations of certain autoantibodies. This situation becomes even more complicated when it is appreciated that certain autoantibodies may precede the underlying disease for many years [11, 12]. Furthermore, some autoantibodies are found in first degree relatives who may not develop disease features even after follow-up [13]. Therefore, when an autoantibody is detected that does not correspond to

*Address correspondence to this author at the Dr. Fooke Laboratorien GmbH, Mainstr. 85, 41469 Neuss, Germany; Tel: +049 2131 4742709; Fax: +049 1212-6 466266866; E-mail: m.mahler.job@web.de

the expected clinical picture, it is unclear if the antibody predicts the long-term diagnosis of the patient, is a coincidental finding in a family member, or is simply a false positive finding. In the following section the clinical significance and usefulness of well established markers is discussed (Table 1).

SLE ASSOCIATED AUTOANTIBODIES

Associations Between Anti-Rib-P and Clinical Features in SLE

The ribosomal phosphoproteins are of interest because, so far, they are the only autoantigens that are targeted by antibodies in autoimmunity, allergy and infectious disease (reviewed in [7, 22-25]). Immunoglobulins of the G isotype specific for the three protein components of the 60S ribosomal subunit, designated P0 (38 kDa), P1 (19 kDa), and P2 (17 kDa) (Rib-P), represent a serological feature of SLE patients (reviewed in [22]). A pentameric complex composed of one copy of P0 and two copies each of P1 and P2 interacts with the 28S rRNA molecule to form a GTPase domain, which is active during the elongation step of protein transla-

tion (reviewed in [22]). The major epitope of the Rib-P autoantigen has been localized to the carboxy terminal domain, which is highly conserved in all three proteins and contains two phosphorylated serine residues (reviewed in [7, 22]) and both acidic and hydrophobic clusters, but not the phosphorylation of the P proteins, are critical for autoantibody binding [26]. Epitope mapping studies have shown that the major epitope domain is located within the last 6 C-terminal amino acids (GFGLFD) [26]. Various techniques, in combination with a variety of different antigens, have been proposed for the detection of anti-Rib-P: immunoblotting with native antigens from different sources, purified or recombinant proteins, synthetic peptides (reviewed in [22, 27, 28]). A recent study has confirmed the high efficiency of the immunoblot technique compared to peptide ELISAs based on the C22 peptide and of a multi-antigen peptide (MAP) construct [28]. An obvious advantage of the immunoblot employing native antigens is that the detection of antibodies to the individual Rib-P proteins P0, P1 and P2 is possible [27, 28]. This feature has been incorporated into Rib-P profile ELISA, which contains the C22 peptide in addition to the recombinant antigens [27]. Thus, the most sen-

Table 1. Overview of Autoantigens Associated with Systemic Autoimmune Diseases

Autoantigen	Disease Association	Nomenclature	Cellular Function of the Complex	First Described	Challenge/Controversy
Scl-70	SSc	III	Topoisomerase I	A	Disease specificity for SSc
CENP-B	ISSc	II	Centromere proteins	B	Disease specificity for SSc
Ro52	SjS	I	RING dependent ligase	[15]	Independent aab system from Ro60 and La
SS-A/Ro60	SjS	I	Phosphoprotein-RNA-polymerase III complex	[15]	Independent aab system from Ro52
SS-B/La	SjS	I	Phosphoprotein-RNA-polymerase III complex	[16]	Independent aab system from Ro52
Jo-1	PM	I	Histidyl-tRNA-synthetase	[17]	Disease specificity for PM
Rib-P	SLE	II	Ribosomal ribonucleoprotein	[14]	Disease specificity for NPSLE
Sm	SLE	I	splicing of hnRNA	C	Selection of antigen (peptide, recombinant protein or native antigen)
RNP	MCTD	II	Ribonucleoprotein, hnRNA splicing	[18]	Selection of antigen (peptide, recombinant protein or native antigen)
RNAP	SSc	II	RNA polymerase, RNA synthesis	D, E	Clinical evaluation of immunoassays with recombinant RNAP
PM/Scl	PM/Scl	III	Polymyositis/scleroderma complex = exosome, RNA processing	[19]	Selection of antigen (peptide, recombinant protein or native antigen)
Ku	SLE, overlap	I	DNA-dependent protein kinase	[20]	Disease association
GWB	SjS, MMSN, SLE	IV	Involved in mRNA processing by siRNA and miRNA pathways	F	Disease association
PCNA	SLE	II	Proliferating cell nuclear antigen/elongation factor of DNA polymerase delta	[21]	Disease association
Fibrillarin	SSc	IV	Component of the small nucleolar RNP (U3 RNP)	G	State of the art method for the detection of AFA

I = Name of the patient providing the prototype serum.

II = Cellular function of the autoantigen.

III = Clinical name of the underlying disease.

IV = Distinctive feature of the autoantigen.

A = [103], B = [4], C = [5], D = [140], E = [141], F = [9], G = [86].

Abbreviations: aab = autoantibody; AFA = anti-fibrillarin antibodies; CENP = centromere protein; GWB = GW bodies; hnRNA = heterogeneous RNA; MCTD = mixed connective tissue disease; miRNA = micro RNA; MMSN = mixed motor/sensory neuropathy; NPSLE = neuropsychiatric SLE; PCNA = proliferating cell nuclear antigen; PM = polymyositis; RNP = ribonucleoprotein; siRNA = silencing RNA; SjS = Sjögren's syndrome; SLE = systemic lupus erythematosus; SSc = systemic sclerosis.

sitive method to detect anti-ribosomal antibodies appears to be the combination of different antigens [27, 29]. The reported prevalence of anti-Rib-P antibodies in SLE ranges from 10-40%, being higher in Asian patients (reviewed in [22, 30]). The variation in the observed frequency may be related to a number of factors but appears to be in large part dependent on patient selection [30]. Anti-Rib-P antibodies were mainly detected in patients during the active phase of SLE and were believed to be correlated with lupus nephritis or hepatitis, although these correlations were not found in all studies (reviewed in [22, 28-35]). Further, anti-Rib-P have been reported to be associated with anti-cardiolipin antibodies [36].

Although known for more than 25 years, anti-Rib-P antibodies have not achieved the attention and clinical impact that anti-Sm or anti-dsDNA antibodies have. This might be attributed to the limited reliability of indirect immunofluorescence assays for the detection of these antibodies, to the limited number of international reference samples and to the relatively late discovery of anti-Rib-P in 1979. In a recent international multi-center study performed by eleven research teams with 947 SLE patients and a broad range of disease controls (n = 1113), the high specificity (99.3%) of anti-Rib-P antibodies was confirmed. Of interest, follow-up of one anti-Rib-P positive RA patient revealed that the patient subsequently developed renal disease and sufficient criteria to be classified as SLE [30]. Based on this and studies showing the high positive predictive value, it has been proposed that, akin to anti-Sm and anti-dsDNA, anti-Rib-P may be considered for inclusion as a criterion for the classification of SLE.

Despite substantial investigation, the relationship between anti-Rib-P and organic central nervous system (CNS) involvement and/or psychosis is still controversial (reviewed in [22, 26, 30, 33, 35, 37-58]). These discrepancies have been attributed to methodological differences in the detection of the antibodies, the difference in the criteria used to clinically define and identify various disease features, the demographics and/or the make-up of the patient cohorts, and the analysis of results (reviewed in [22, 34, 35]). A recent meta-analysis performed in 14 research teams by Karassa and colleagues has shown low sensitivity and specificity and thus limited diagnostic value of anti-Rib P for neuropsychiatric SLE (NPSLE) [38]. When the Pubmed data bank was screened to evaluate the studies focussing on the association of anti-Rib-P with NPSLE (keywords: SLE, ribosomal P, CNS in different combinations), 23 studies were identified of which 13 supported this sero-clinical association. The diverse findings of the association of anti-Rib-P antibodies and CNS involvement are summarized in Table 2.

Anti-Nucleosomes (Chromatin)

The prevalence of anti-nucleosomal antibodies (ANuA) in SLE sera is reported to be approximately 60% [60-71]. Several studies analysed the diagnostic accuracy and clinical relevance of ANuA using different antigen sources and detection methods, and their relationship to anti-dsDNA antibodies [60-71]. Some studies reported that ANuA assays had a higher sensitivity than anti-dsDNA for SLE while other studies did not confirm this finding [61, 69]. Moreover, the association between ANuA and disease activity and organ

damage, such as renal crisis, has also been controversial [66, 69]. Although ANuA were widely considered specific for SLE and drug-induced lupus [70], a high prevalence of ANuA was also reported in a cohort of scleroderma patients [71]. This lack of clinical specificity might be attributed to the antigen used for the nucleosome immunoassay or by the cut-off of the assay used [67]. In a recent study, it was shown that nucleosomes stripped of certain proteins (e.g. histone H1 and high mobility group proteins) yield significantly higher disease specificity than pure nucleosomes [60]. Table 3 presents an overview of studies on ANuA in SARD.

Anti-Sm/RNP

Antibodies directed to Sm were first described by Tan and Kunkel in 1966 and are found in 5-30% of patients with SLE depending on the detection system and the ethnicity of the SLE population being studied [5, 6]. As a hallmark in the diagnosis of SLE, they have been included as one of the SLE classification criteria of the American College of Rheumatology [72]. The Sm antigen is part of the spliceosomal complex that catalyzes the splicing of hnRNA and is composed of at least nine different polypeptides with molecular weights ranging from 9 – 29.5 kDa (B1, B', B3, D1, D2, D3, E, F and G). All of these core proteins, but most frequently the B and D polypeptides, are targets of the anti-Sm autoimmune response [73, 76, 80]. However, since SmBB' and U1 specific RNPs share the cross-reactive epitope motif PPPGMRPP in MCTD patients, SmD is regarded as the most SLE specific Sm antigen [74]. Within the SmD autoantigen family, reactivity with SmD1 and D3 is more common than D2 recognition with a pronounced immuno-reactivity to SmD1 [75]. In epitope mapping studies of SmD1 and BB', the major reactivity was predominantly found in the C-terminal domains [76]. Recently, it was shown that snRNP proteins, such as SmD1, D3, and BB', contain symmetrically dimethylated arginine (sDMA) and that these residues constitute major epitopes on SmB and SmD polypeptides [77-79]. Several immunoassays designed for research studies and diagnostic laboratory use, have been developed [79, 80-82]. The antigens employed in these tests included purified native proteins, recombinant polypeptides or synthetic peptides [79, 80, 82] and a high degree of clinical accuracy has been reported for immunoassays based on the SmD peptides (SmD1 83-119 and SmD3 108-122) [80-82]. The SmD1 peptide has been shown to be dependent on casein as a cofactor for antibody binding [83] and the SmD3 peptide contains an sDMA residue as the key amino acid [79]. Surprisingly, the prevalence of anti-SmD1 83-119 was reported as high as 70% in SLE and up to 20% in related SARD, which is not in agreement with the known prevalence of anti-Sm antibodies. A recent investigation by Dieker and colleagues questioned the specificity of the SmD1 assay since SmD1 83-119 showed a pronounced binding of dsDNA suggesting a putative bridging effect [84]. This suggestion is supported by the observation that both the prevalence and the titer of anti-SmD1 83-119 are significantly higher in an anti-dsDNA positive serum group than in an anti-dsDNA negative group [82]. Another explanation might be the selection of a low cut-off value for the SmD1 test as around 20% of other SARD also showed anti-SmD1 83-119 reactivity in this study [82]. Further studies are under way to shed more light on this controversy.

Table 2. Association Between Anti-Rib-P and CNS Involvement in SLE

Association with CNS Disease	No. of Patients	Comment	Antigen/Assay	Reference
Yes	20 LP	Association between anti-Rib-P and disease activity in two patients	C22/RIA	A
No	2	Small number of samples		B
Yes	269 SLE 82 NPLE	Strong association with severe depression and NPLE, but not with non-psychiatric neurologic disease	C22/ELISA	C
No	54 SLE with ME	16 positive among 946 for which a anti-dsDNA test was requested. 12/12 samples from which clinical data was available had SLE	C22/ELISA N/IB	D
Yes	91 SLE	High prevalence 41%; The occurrence of NPLE was significantly higher in patients with anti-P than in those without anti-P (9/38 vs 1/53).	N/IB	E
Yes	66 SLE 52 NPLE	Association between anti-Rib-P and diffuse NPLE (with primarily psychiatric disease)	unclear	F
No	51 SLE	Association between anti-Rib-P and either psychological or cognitive abnormalities	C22/ELISA	G
Yes	70 SLE	No association between anti-Rib-P and NPLE, but with CNS in the absence of LP, samples from active Japanese SLE	RP0/ELISA	H
Yes	79 SLE 13 NPLE	Psychoses in active phase, decreased during remission	unclear	I
Yes	394 SLE	Association between anti-Rib-P and diffuse neuropsychiatric dysfunction (depression and LP)	RP2/ELISA	J
Yes	178 SLE 28 NPLE	Association between anti-Rib-P and CNS involvement particularly in patients without anticardiolipin antibodies	C22/ELISA	K
No	149 SLE	No association between anti-Rib-P and NPLE, but with presents of anticardiolipin	MAP C10/ELISA	L
No	94 SLE	No significant correlation of anti-Rib-P with central nervous system manifestations or renal involvement in SLE patients	C22/ELISA	M
No	289 SLE	Association between anti-Rib-P and haemolytic anaemia, alopecia	C22/LIA	N
No	947 SLE	International multi-center study; Association between anti-Rib-P and NPLE only in some participating centers	RP0, P1, P2/ELISA	O
No		Association between anti-Rib-P and neuropsychiatric disorders (psychiatric and neurological), but not with CI	C22/ELISA	P
No	70 SLE 15 CI	No association between anti-Rib-P and CI	NP/ELISA	Q
Yes	75 SLE, 28 LP, 21 NPLE	Association between anti-Rib-P with lupus psychosis. Difference to previous studies was speculated to be related to the purity of the antigen.	C22-HSA conjugate/ELISA	R
Yes	70 SLE	Association between anti-Rib-P (recombinant P0) and NPLE but not between C22 or C22 truncated recombinant P0. Difference to previous studies was speculated to be related to the nature of the antigen.	RP0, C22/ELISA	S
Yes	144 SLE	Anti-Rib-P titer was significantly higher in 12 patients with LP	NP/ELISA	T
Yes	141	Only two patients had LP	N/DID or WB + ELISA	U
No	51	Association between NPLE and AECA	RP0, P1, P2/ELISA	V
Yes	70	The frequency of CSF anti-Rib-P was significantly higher in patients with NPLE	WB rat liver ribosomes	W

Abbreviations: AECA = anti-endothelial cell antibodies; CI = cognitive impairment; CSF = cerebrospinal fluid; CNS = central nervous system; DID, double immunodiffusion; ELISA = enzyme linked immunoassay; HSA = human serum albumin; IB, immunoblot; ME, monosymptomatic exacerbations, N, native; NP, native protein; NPLE = neuropsychiatric lupus erythematosus; LP = lupus psychoses; RIA, radioimmunoassay; RP0, recombinant protein 0; RP2, recombinant protein 2; SLE = systemic lupus erythematosus; WB, western blot.

A = [7]; B = [39]; C = [48]; D = [40]; E = [41]; F = [42]; G = [50]; H = [52]; I = [43]; J = [53]; K = [44]; L = [45]; M = [26]; N = [37]; O = [28]; P = [57]; Q = [49]; R = [58]; S = [55]; T = [46]; U = [32]; V = [47]; W = [54].

SSC ASSOCIATED AUTOANTIBODIES

Anti-Fibrillar Antibodies (AFA)

In 1985, fibrillar, a key component of the U3-RNP complex was isolated by immunoprecipitation (IP) by Ochs

and colleagues using human antibodies that produced a characteristic 'clumpy' nucleolar pattern of staining on a variety of tissue culture cells (reviewed in [85, 87]). Anti-fibrillar antibodies (AFA) were found to stain not only the nucleoli, but also Cajal bodies and portions of condensed chromatin in

Table 3. Clinical Value of Anti-Nucleosome/Chromatin Antibodies

SLE (n)	Controls (n)	ANuA > Anti-dsDNA	Sensitivity %	Specificity %	Sero-Clinical Assessment	Method	Comment	Reference
101	233	yes	86.1	95.3	No	ELISA	No monitoring, high prevalence in SSc (22.8%) but low titers	A
70	70	no	87.0	58.0	nd	ELISA	AUC of ANuA higher than of anti-dsDNA, but a selected cut-off sensitivity similar	B
295	220	nd	62	48	nd	ELISA	Crude (unstripped) nucleosomes; Specificity calculated based on SSc controls (n = 119)	C
295	220	nd	58	100	nd	ELISA	Pure (stripped) nucleosomes; Specificity calculated based on SARD controls (n = 220)	C
305	530	no	30	90	nd	ELISA	Good correlation between dsDNA ANuA, no additional information for the diagnosis of SLE	D
84	/	nd	78.6	nd	yes	DB	5/21 (23.8%) dsDNA neg have ANuA; Renal disorder (p = 0.04) and ECLAM (p = 0.002) was higher in ANuA positive patients	E
87	/	yes	40.0-58.6	nd	yes	ELISA	Sensitivity in SLE; ANuA more sensitive, but less specific than anti-dsDNA antibodies to active SLE and active nephritis	E

Abbreviations: ANuA = anti-nucleosome antibodies; AUC = area under the curve; DB = dot blot; ECLAM = European Consensus Lupus Activity Management; ELISA = enzyme linked immunoassay; nd = not determined; SARD = systemic autoimmune rheumatic diseases; SLE = systemic lupus erythematosus. A = [63]; B = [65]; C = [60]; D = [69]; E = [61]; F = [64].

metaphase cells, a feature which is consistent with the known cellular distribution of the U3-RNP particle (reviewed in [85, 88]). Human fibrillarin is a 34 kDa protein (320 amino acids) composed of three domains: the N-terminal dimethylarginine rich domain, the central domain that putatively binds RNA, and the C-terminal domain, which has not yet been attributed a defined cellular function [88].

The prevalence of AFA in patients with SSc varies between 4% and 14% depending on the origin of the patients and the detection assays [89-94]. Studies to date agree that in human sera AFA are present independent of anti-CENP, anti-Scl-70, U1-RNP or anti-RNA polymerase antibodies (RNAP), but are frequently accompanied by anti-MPP10, anti-hU3-55k protein and other antibodies directed to other components of the U3-RNP complex (reviewed in [85, 95]). The frequency of AFA is remarkably higher in patients of African descent with SSc where it is reported to be as high as 16–22% (reviewed in [85, 89-91]). However, as this antibody occurs less frequently than the major SSc-specific autoantibodies, most reports involved only small numbers of patients [89-94, 96]. The lack of a reliable high throughput method (e.g. an ELISA) for the detection of anti-fibrillarin antibodies has been a very significant limitation to the generation of comprehensive data on AFA since time consuming methods are still routinely used for the detection of AFA [96]. The presence of AFA in Caucasian patients with SSc was associated with diffuse skin involvement, but this correlation is not nearly as strong as with anti-topo-I antibodies [94, 97].

Independent studies have suggested that AFA may be associated with pulmonary arterial hypertension (PAH) and skeletal muscle disease, especially in young African-American males, although AFA did not predict PAH in pa-

tients with ISSc (reviewed in [85]). Interestingly, considering the dominant clinical pattern of internal organ involvement in diffuse scleroderma, AFA have not been associated with a higher mortality rate, although those who died tended to succumb to PAH (reviewed in [85]). Several studies have suggested a triggering effect of xenobiotics, such as mercury on the generation of AFA (reviewed in [98-102]). This idea is supported by the observation that several anti-fibrillarin specific monoclonal antibodies could be generated from certain strains of mice exposed to mercury and other heavy metals and some AFA positive SSc patients have significantly higher urinary mercury levels compared to other SSc patients and controls [101]. Since there are efforts to reduce the heavy metal exposure in the environment, one might speculate that the frequency of AFA will also decrease. Further studies that address the possibility that heavy metals and other xenobiotics are related to the induction of the AFA response are required to understand this relationship.

Anti-Scl-70

Historically, anti-Scl-70 (topo-I) antibodies were regarded as a highly specific marker for SSc, being found in about 20-40% of SSc patients (reviewed in [85, 103]). The terminology Scl-70 was based on early studies indicating that the target antigen was 70 kDa [103], but later evidence indicated that the target antigen DNA topoisomerase I (topo-I) was 110 kDa [104, 105]. Hence, the preferred nomenclature is topo-I, although the Scl-70 terminology persists in the literature.

Anti-topo-I antibodies were most commonly associated with the diffuse form of SSc attended by progressive disease and high mortality rates that were often attributed to right heart failure in association with pulmonary fibrosis and re-

strictive lung disease [106]. One study of Japanese patients with SSc suggested that anti-topo I was associated with renal crisis [107], but no convincing association with this feature has been found in other investigations. The presence of anti-Scl-70 antibodies in a patient initially evaluated for Raynaud's phenomenon has been shown to predict the future development of SSc [108]. Anti-CENP and anti-topo-I antibodies are very rarely found in the same serum, being present in less than 0.5% of a cohort of SSc patients [109].

A controversy arose when studies indicated that up to 25% of SLE patients without any clinical evidence of SSc had anti-topo-I antibodies [110-114] (Table 3). There have been attempts to reconcile these data with earlier data on the basis of the sensitivity and specificity of the various assays used for these studies. One of the potentially confounding features of anti-topo-I antibodies is that topoisomerase is a DNA binding protein and some investigators have considered that anti-DNA/DNA complexes in sera of SLE patients might bind to topo I resulting in a false positive result. Another explanation is that this might be attributed to the antigen used in the immunoassay since a study that used recombinant topo-I expressed in insect cells found that anti-topo-I antibodies are highly specific for SSc and not found in SLE [115]. The unanticipated binding characteristics of topo-I are emphasized in a recent study showing that topo-I bound to the cell surface of fibroblasts in a dose-dependent and saturable manner, where it was recognized by anti-topo-I from SSc patients [116]. The nature of the topo-I binding site is of interest since it may point to a mechanism that underlies the amplification of the fibrogenic cascade in anti-topo-I-positive patients. Of interest, over two decades ago there was interest in cell surface DNA and DNA receptors [117], historical studies that may bear on these most recent observations of topo-I.

Anti-CENP

Sera from patients suffering from the limited form of SSc (lSSc; also referred to as CREST = *calcinosis cutis*, *Raynaud's phenomenon*, *esophageal dysmotility*, *sclerodactyly*, *telangiectasia* variant) frequently contain anti-CENP antibodies, which are predominately directed against three autoantigens: CENP-A (17-19.5kDa), CENP-B (80kDa), and CENP-C (140kDa) [3, 4]. Other CENP targets include CENP-D, CENP-E, and CENP-F, although they are found in

different conditions and CENP-D has yet to be characterized (reviewed in [85, 122, 123, 125]). Most notably, up to 50% of patients with anti-CENP-F were found to have a malignancy [125]. In IIF studies on HEp-2 cells, anti-CENP-A, -B, -C antibodies produce a speckled staining pattern in the interphase nucleus and metaphase chromatin corresponding to the centromere domains [3, 4]. Based on the structural homology and on functional studies, CENP-A is thought to replace histone H3 in a distinct nucleosome-like structure (reviewed in [122]). CENP-A is essential for kinetochore formation and function, and may serve as an epigenetic mark that propagates centromere identity through replication and cell division (reviewed in [122]). Historically, anti-CENP antibodies have been considered a relatively specific marker for lSSc (reviewed in [3, 85]). Since these initial studies, the clinical associations of anti-CENP have widened. One recent study confirmed the disease specificity of anti-CENP antibodies while two other studies using different technologies including different LIA and addressable laser bead assays (ALBIA) have reported ACA in other systemic rheumatic diseases such as SLE (1.8 – 5.6%) or RA (2.2%) [118-131]. The anti-CENP reactivity in RA patients might be attributed to cross-reactive antibodies that react with both CENP-B and heterogeneous nuclear RNP (hnRNP) [132] or to the CENP-A epitope motif G/A-P-R/S-R-R (reviewed in [133, 134-136]). Mimotopes of this motif were found in a number of autoantigens and in the Epstein-Barr nuclear antigen 1 (EBNA-1) [136]. In a similar approach, it has been shown that autoantibodies directed to the epitope motif G/A-P-R/S-R-R cross-react with several mimotopes. However, these mimotopes often represent cryptic epitopes, explaining in part the obvious conflict with the known high specificity of anti-CENP-A autoantibodies [6]. In a follow-up study of one patient with SSc, it was suggested that anti-CENP-A peptide antibodies may be induced by inter- and intermolecular epitope spreading from histone protein H3 and that anti-CENP-A antibodies are detectable earlier than antibodies to CENP-B [137]. Therefore, CENP-A antibodies may be a promising early marker for the diagnosis of SSc.

Moreover, a difference in the reactivity to CENP could be found in patients with SSc, SjS or other autoimmune diseases [138]. One of the cautions in attributing anti-CENP antibodies to a variety of SARD is that it is well established that lSSc tends to progress slowly and it may be 25 years (or

Table 4. Sensitivity and Specificity of Scl-70 (Topo I) Autoantibodies

n pos (% pos)		Antigen	Method	Company	Reference
SLE	SSc				
113 (12.7%)	8/43 (18.6)	np	ALBIA	AtheNA Multi-Lyte [®] , Zeus Scientific, Inc. Raritan, NJ, USA	A
32/125 (25%)	16/89 (18%)	R	ELISA	Varelisa [®] , Phadia, Freiburg, Germany	B
289 (2.6%)	/	R	LIA	INNO-LIA [™] ANA Update, Innogenetics, Gent, Belgium	C
4/332 (1.2%)	8/19 (42%)	R	ALBIA	BioPlex [™] 2200 ANA Screen kit, Bio-Rad Laboratories, Hercules, CA, USA	D
113 (4.7%)	/	np	ALBIA	AtheNA Multi-Lyte [®] , Zeus Scientific, Inc. Raritan, NJ, USA	E
0/57 (0.0%)	/	R	LIA	recomLine ANA, Mikrogen, Neuried, Germany	F
1/57 (1.8%)	/	R	LIA	INNO-LIA [™] ANA Update, Innogenetics, Gent, Belgium IMTEC-ANA-LIA, ImTec, Berlin, Germany	F

Abbreviations: ALBIA = addressable laser bead assay; ELISA = enzyme linked immunoassay; LIA = line immunoassay; np = not provided = R = recombinant; SLE = systemic lupus erythematosus; SSc = systemic sclerosis.

A = [119]; B = [110]; C = [37]; D = [115]; E = [111]; F = [118].

longer) until the spectrum of clinical features of ISSc (i.e. CREST) or other conditions are clinically evident [139]. Long term, longitudinal studies are needed before the full picture of clinical features associated with anti-CENP, or any autoantibody, is clearly defined.

Anti-RNAP

Autoantibodies to RNA polymerase (RNAP) were first reported in 1982 [140] but it was not known until 1987 that RNAP-I was identified as one of the molecular targets [141]. Following these seminal studies, it was found that RNAP-III was the target autoantigen in approximately 20% of SSc sera and was associated with diffuse SSc and an increased risk of heart and lung involvement [142-148]. In contrast to the high specificity of anti-RNAP-I and -III, antibodies to RNAP-II have been reported in other disease conditions such as SLE/SSc overlap syndrome (reviewed in [85]).

Despite the evidence that antibodies to RNAP-III are found in approximately 20% of SSc, a frequency that approximates the frequency of antibodies to topo-1 (Scl-70) and CENP, the detection of anti-RNAP relied on time consuming IP and radioimmunoprecipitation (RIP) assays. However, in the last few years, ELISAs that employ a recombinant RNAP-III fragment [149] have been described and have been employed in studies to evaluate the clinical and serological parameters associated with these autoantibodies [150, 151]. The observation that RNAP-I antibodies nearly always coexist with RNAP-III antibodies [142, 144, 151] has led to the development and marketing of at least two commercial ELISA kits. As with other assays and kits developed using dominant epitopes represented as peptide fragments, it will be important to validate the disease specificity and sensitivity in laboratories in various geographic and genetic environments.

Another challenge in the identification of anti-RNAP is that early studies suggested that these autoantibodies were correlated with a speckled nucleolar staining pattern as detected by IIF. However, a recent study found that RNAP-III antibodies were not consistently associated with this nucleolar IIF pattern on conventional HEP-2 cell substrates but more consistently had a nuclear speckled IIF pattern [151]. This finding is consistent with observations that monoclonal anti-RNAP-III antibodies stain nuclei whereas antibodies to RNAP-I stain nucleoli [141]. Therefore, it cannot be assumed that IIF on conventional HEP-2 substrates serves as a useful screen to detect these antibodies. This presents a challenge to the clinical laboratory that might have relied on the IIF staining pattern to triage sera that would then be tested by a more specific ELISA, such as the RNAP-III ELISA kits that have recently been released on the market. Of interest, this may represent an advantage of multiplexed diagnostic platforms such as ALBIA where multiple autoantibodies can be detected in a single specimen [152].

OTHER AUTOANTIBODIES

Anti-Jo-1

Traditionally, anti-Jo-1 (histidyl tRNA synthetase; HRS) antibodies have been reported as specific marker for patients with polymyositis (reviewed in [153, 156]). The techniques used to detect these antibodies include ELISA, line immuno-

assays, immunoblotting and multiplex systems (reviewed in [154, 157, 158]). Some studies have shown that subsets of PM patients with anti-Jo-1 frequently have interstitial lung disease and pulmonary fibrosis, non-erosive arthritis, and Raynaud's phenomenon [159]. Furthermore, patients with Jo-1 syndrome have been reported to have overlapping features of seronegative RA, SjS with anti-SS-A/-B antibodies; SSc and bronchial carcinoma with anti-CENP antibodies and glucocorticoid-sensitive psychosis [160-162]. The clinical picture of anti-Jo-1 positive patients is variable and most patients had features other than myositis at disease onset [161]. Therefore, in early or subclinical forms of the disease, overt inflammatory muscle disease may be absent leading the clinician to conclude that a positive anti-Jo-1 result is a non-specific finding. The information that anti-Jo-1 antibodies are markers of specific disease subsets of inflammatory muscle disease, specifically PM, aids the development of better disease definitions and likely has implications on the clinical management of these patients [163]. In addition, long term longitudinal studies of patients with Jo-1 antibodies without clinical evidence of muscle disease are required to determine if such antibodies can be transiently expressed and if not, the prognostic significance of this autoantibody in such a clinical setting.

Anti-Alpha-Fodrin

In 1997 Haneji and colleagues identified the 240 kDa alpha-fodrin as a putative autoantigen in a mouse model of SjS and showed that it is cleaved by the enzyme caspase 3 into smaller fragments of 150 and 120 kDa (reviewed in [164, 165]). Subsequently, several studies reported IgA and IgG anti-fodrin antibodies in 88% and 64% of SjS patients, respectively (reviewed in [164]). Anti-fodrin antibodies were detected in serum, tears, and saliva from patients with SjS [166]. Since the identification of the antigen, several studies have investigated the clinical prevalence and usefulness of anti-fodrin IgG and IgA antibodies in SjS with conflicting findings. The wide difference in the prevalence of anti-fodrin antibodies from the first reports to these subsequent reports was attributed to the patient selection and therapies (reviewed in [164]). A significantly higher prevalence of anti-fodrin was found in a SjS cohort selected on the basis of the San Diego criteria [167] compared to groups defined on the basis of the European Community Study Group criteria (reviewed in [168, 169]), or the American-European Consensus group criteria (reviewed in [164]). A clinical finding that requires confirmation is the observation that anti-fodrin antibodies represent a marker for SjS patients suffering from neurological disorders (reviewed in [164, 170]).

Epitope analysis of anti-fodrin antibodies revealed that patients with primary and secondary SjS show a distinct epitope recognition pattern and it was suggested that a recombinant expression fragment of alpha-fodrin might be a useful serological marker to differentiate between primary and secondary SjS [171]. Recently, an alpha-fodrin derived peptide (RQKLEDSYRFQFFQRDAEEL) was identified as a sensitive and specific marker targeted by autoantibodies in patients with primary SjS and to a lesser extent in secondary SjS, SLE and RA [172]. Studies of larger patient cohorts are required to validate the proposed usefulness of specific epitopes and peptides as diagnostic markers for SjS.

Anti-Ro52 and Ro60

Antibodies to the SS-A/SS-B antigens were first described in SjS and SLE patients, but have later been reported in patients with SSc, RA, subacute cutaneous lupus, neonatal lupus syndrome and DM (reviewed in [173]). Historically, the SS-A/SS-B system was reported as a heterogeneous antigenic complex, comprised of three different proteins (SS-A 52 kDa Ro, SS-A 60 kDa Ro and SS-B La) and one of four small RNAs molecules (reviewed in [173, 174]). One of the confounding issues was that the function of Ro52 was unknown. However, it was recently identified as a RING dependent E3 ligase that is over-expressed in peripheral blood mononuclear cells in SjS and SLE patients and this leads to reduced cell growth and apoptosis [176, 177]. Clinically, it had been shown that Ro52 was associated with neuromuscular disease such as myositis, sensory and motor neuropathy, while Ro60 was associated with SLE and SjS. A strong association of anti-Ro52 with anti-Jo-1 antibodies has also been reported [178, 179]. Based on these recent findings, it could be concluded that the functional and immunological association of Ro52 with SS-A/Ro60 or SS-B/La is less significant than initially reported [176, 177, 180]. It follows that the traditional terminology of the Ro autoantigens should be changed accordingly. Since the Ro52 antigen is not uniformly associated with SjS, it should be referred to as Ro52 rather than SS-A/Ro52, whereas the 60 kDa antigen could be referred to as Ro60 or SS-A/Ro60. In addition, the practice of combining Ro52 and SS-A/Ro60 protein in single diagnostic assays should be discontinued and anti-SS-A/Ro60 and anti-Ro52 antibodies should be tested separately [180].

CONCLUSION

Despite many years of research on autoantibodies associated with SARD several issues are still controversial. The reasons for the controversies are diverse, but include the difference in the patient selection, the lack of standardization for autoantibody assays and the genetic background of the patient cohorts. Further prospective studies with a unique study protocol are required to evaluate the exact clinical relevance of an autoantibody assay. Multi-center studies with larger patient cohorts and appropriate disease control groups, as well as an unique study protocols is a major requirement and challenge if the clinical impact of autoantibodies that occur infrequently is going to be more clearly defined and understood.

ABBREVIATIONS

ALBIA	=	Addressable laser bead assay
AFA	=	Anti-fibrillarin antibodies
ANuA	=	Anti-nucleosome antibodies
CENP	=	Centromere proteins
CI	=	Cognitive impairment
CNS	=	Central nervous system
CREST	=	Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia
CSF	=	Cerebrospinal fluid
DB	=	Dot blot
DID	=	Double immunodiffusion

DM	=	Dermatomyositis
ELISA	=	Enzyme linked immunoassay
GWB	=	GW bodies
IIF	=	Indirect immunofluorescence
LIA	=	Line immunoassay
ISSC	=	Limited systemic sclerosis
MCTD	=	Mixed connective tissue disease
miRNA	=	Micro RNA
NPSLE	=	Neuro-psychiatric systemic lupus erythematosus
PAH	=	Pulmonary arterial hypertension
PM	=	Polymyositis
RA	=	Rheumatoid arthritis
RIA	=	Radioimmunoassay
RNAP	=	RNA polymerase
RNP	=	Ribonucleoprotein
SARD	=	Systemic autoimmune rheumatic diseases
siRNA	=	Silencing RNA
SjS	=	Sjögren Syndrome
SLE	=	Systemic lupus erythematosus
SSc	=	Systemic sclerosis

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