Original Article

Evaluating Anti-SmD1-amino-acid 83-119 Peptide Reactivity in Children with Systemic Lupus Erythematosus and Other Immunological Diseases

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Abstract

Background: SmD1-amino-acid 83-119 peptide ($SmD1_{83-119}$) is the major epitope of Smith (Sm) antigen, which is specific for adult systemic lupus erythematosus (SLE). The anti-$SmD1_{83-119}$ antibody has exhibited higher sensitivity and specificity than anti-Sm antibody in diagnosing adult SLE. However, the utility of anti-$SmD1_{83-119}$ antibodies remains unclear in children with SLE (cSLE). This study aimed to assess the characteristics of anti-$SmD1_{83-119}$ antibody in the diagnosis of cSLE.

Methods: Samples from 242 children with different rheumatological and immunological disorders, including autoimmune diseases (SLE [n = 46] and ankylosing spondylitis [AS, n = 11]), nonautoimmune diseases (Henoch-Schonlein purpura [HSP, n = 60], idiopathic thrombocytopenia purpura [n = 27], hematuria [n = 59], and arthralgia [n = 39]) were collected from Shanghai Children's Medical Center from March 6, 2012 to February 27, 2014. Seventy age- and sex-matched patients were enrolled in this study as the negative controls. All the patients' sera were analyzed for the anti-$SmD1_{83-119}$, anti-Sm, anti-U1-nRNP, anti-double-stranded DNA (dsDNA), anti-nucleosome, anti-SSA/Ro60, anti-SSA/Ro52, anti-SSB, anti-Scl-70, and anti-histone antibodies using the immunoblotting assay. The differences in sensitivity and specificity between anti-$SmD1_{83-119}$ and anti-Sm antibodies were compared by Chi-square test. The correlations between anti-$SmD1_{83-119}$ and other auto-antibodies were analyzed using the Spearman's correlation analysis. A value of $P < 0.05$ was considered statistically significant.

Results: Thirty-six out of 46 patients with cSLE were found to be positive for anti-$SmD1_{83-119}$, while 12 patients from the cSLE cohort were found to be positive for anti-Sm. Compared to cSLE, it has been shown that anti-$SmD1_{83-119}$ was only detected in 27.3% of patients with AS and 16.7% of patients with HSP. In comparison with anti-Sm, it has been demonstrated that anti-$SmD1_{83-119}$ had a higher sensitivity (78.3% vs. 26.1%, $\chi^2 = 25.1$, $P < 0.05$) and a lower specificity (90.8% vs. 100%, $\chi^2 = 13.6$, $P < 0.05$) in the diagnosis of cSLE. Further analysis revealed that anti-$SmD1_{83-119}$ antibodies were positively correlated with anti-dsDNA, anti-nucleosome, and anti-histone antibodies in cSLE. Moreover, it has been clearly shown that anti-$SmD1_{83-119}$ was more sensitive than anti-Sm in discriminating autoimmune diseases from nonautoimmune disorders in patients with arthralgia or hematuria.

Conclusions: Measurement of anti-$SmD1_{83-119}$ in patients with cSLE has a higher sensitivity and a marginally lower specificity than anti-Sm. It has been suggested that inclusion of anti-$SmD1_{83-119}$ testing in the integrated laboratory diagnosis of cSLE may significantly improve the overall sensitivity in child populations.

Key words: Autoantibodies; Children; Diagnosis; SmD1-amino-acid 83-119 Peptide; Systemic Lupus Erythematosus

Introduction

Anti-Smith (Sm) antibody, which was first described by Tan and Kunkel,[1] is specific for adult systemic lupus erythematosus (SLE) according to the American College of Rheumatology.[2] However, the frequency of anti-Sm antibody in adult SLE patients is low (ranging from 5% to 30%) depending on the serologic tests used and the ethnic

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origin of the patient population.[3,4] More researchers have exploited various techniques, such as immunoblotting, immunofluorescence, and enzyme-linked immunosorbent assay (ELISA), to increase anti-Sm antibody detection sensitivity.[3,5-8]

Sm antigen is composed of at least nine different polypeptides (SmB1, SmB', SmB3, SmD1, SmD2, SmD3, SmE, SmF, and SmG);[5] these proteins, especially SmB and SmD1 peptides, are targets for anti-Sm antibody. Because SmBB' and U1-nRNP share a cross-reactive epitope, the SmD1 protein is likely the most important polypeptide in the Sm antigen.[9] Among SmD1 peptides, the SmD1-amino-acid 83-119 peptide ($SmD1_{83-119}$), which was discovered through epitope mapping using 13-mer peptides overlapping by ten amino acids and sera from adult SLE patients, was described as a major epitope targeted by autoantibodies in adult SLE sera.[10] Researchers further revealed that the polypeptide was a conformational epitope that was not accessible in the full-size SmD1 protein. Compared with Sm antigen, $SmD1_{83-119}$ peptide may produce higher sensitivity (36–70%) when it is used as the ELISA antigen for the detection of anti-$SmD1_{83-119}$ in adult SLE without significantly compromised specificity.[11]

Approximately 15–20% of adult patients with SLE exhibit some laboratory features of SLE in childhood and adolescence.[12,13] Up to date, $SmD1_{83-119}$ peptide has not been fully characterized in children with SLE (cSLE). The aim of the present study was to evaluate the sensitivity and specificity of anti-$SmD1_{83-119}$ autoantibody detection in cSLE. In addition, we also intended to reveal the correlation between anti-$SmD1_{83-119}$ antibody and other autoantibodies in cSLE.

Methods

Serum samples

Samples from 242 children with autoimmune diseases and nonautoimmune diseases were collected continuously from Shanghai Children's Medical Center (from March 6, 2012 to February 27, 2014), including cSLE (n = 46), ankylosing spondylitis (AS, n = 11), Henoch-Schonlein purpura (HSP, n = 60), idiopathic thrombocytopenia purpura (ITP, n = 27), hematuria (n = 59), and arthralgia (n = 39) patients. Moreover, seventy age- and sex-matched healthy children were enrolled in this study as the negative controls. The cSLE patients were diagnosed using the American College of Rheumatology's SLE criteria.[2] Patients with hematuria and arthralgia were also included in the study due to the fact that these patients were suspected of autoimmune disorders on their first visit to the clinic but at the time of sample collection their autoimmune disorders were still not finally established. All sera were stored at −80°C until use. All enrolled patients and healthy children were from the Shanghai Children's Medical Center (from March 6, 2012, to February 27, 2014). Written informed consent was obtained from the parents or guardians of all patients and healthy children before the serum was collected. This study was approved by the Shanghai Children's Medical Center's Ethics Committee.

Immunoblotting analysis

Immunoblotting analyses for anti-$SmD1_{83-119}$, U1-nRNP, SSA/Ro52, SSA/Ro60, SSB, Scl-70, double-stranded DNA (dsDNA), nuclearsome, and histone antibodies (IMTEC Immundiagnostika GmbH, Berlin, Germany) as well as anti-Sm antibody (EUROIMMUN Medizinische Labordiagnostika AG, Lubeck, Germany) were performed in accordance with the manufacturer's instructions. The IMTEC $SmD1_{83-119}$ peptide contains the symmetrical dimethylarginine modification described by Mahler *et al*.[14] The EUROIMMUN native Sm antigen was purified from bovine thymus and spleen extracts using affinity chromatography. In brief, after preincubating with blocking buffer, the strip coated with the $SmD1_{83-119}$ peptide and additional 11 auto-antigens was incubated with sera diluted at 1:100 in blocking buffer. The antigen-antibody complex was immunostained using alkaline phosphatase-conjugated anti-human-IgG secondary antibodies. The bands were visualized after adding the substrate, and bands that were clearly visible compared with the control bands were considered positive.

Statistical analysis

We compared the sensitivity and specificity between anti-$SmD1_{83-119}$ and anti-Sm antibodies using Chi-square test. We analyzed the correlation between anti-$SmD1_{83-119}$ antibody and other auto-antibodies, such as anti-Sm antibodies and anti-dsDNA antibodies, using the Spearman's correlation analysis. All statistical analyses were performed using the SPSS 19.0 software (IBM Corporation, Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant.

Results

High prevalence of anti-SmD1-amino-acid 83-119 peptide antibody in children with systemic lupus erythematosus

One hundred and seventeen samples from cSLE patients (n = 46) or children with AS (n = 11) and HSP (n = 60) were analyzed using an immunoblotting assay to generate autoantibody profiles. As shown in Table 1, 36 out of 46 patients with cSLE were positive for anti-$SmD1_{83-119}$, while 12 out of the 46 patients were positive for anti-Sm. Furthermore, we observed that 21 out of the 46 cSLE patients exhibited anti-dsDNA reactivity, which indicates a higher anti-$SmD1_{83-119}$ prevalence in cSLE than anti-dsDNA antibodies. In addition, we also observed positivity for anti-$SmD1_{83-119}$ antibody in three out of 11 patients with AS and in 10 out of 60 patients with HSP; however, none from AS and HSP showed reactivity to Sm antigen.

Sensitivity and specificity of anti-SmD1-amino-acid 83-119 peptide antibody in children with systemic lupus erythematosus and nonsystemic lupus erythematosus

As shown in Table 2, anti-$SmD1_{83-119}$ antibody exhibited higher sensitivity than anti-Sm antibody for cSLE detection (78.3% vs. 26.1%, $\chi^2 = 25.1$, $P < 0.05$). In comparison, 3 of 11 AS patients (27.3%) and 10 of 60 HSP patients (16.7%) exhibited anti-$SmD1_{83-119}$ antibody in sera. Furthermore, none of the control children's sera reacted with the $SmD1_{83-119}$ peptide. However, our results also indicated that anti-$SmD1_{83-119}$ antibody exhibited compromised specificity compared with anti-Sm antibody in cSLE (90.8% vs. 100%, $\chi^2 = 13.6$, $P < 0.05$).

Correlation between anti-SmD1-amino-acid 83-119 peptide positivity and other autoantibodies in children with systemic lupus erythematosus

The correlations between anti-$SmD1_{83-119}$ antibody and other autoantibodies were investigated using 46 cSLE patients. As shown in Table 3, anti-$SmD1_{83-119}$ reactivity positively correlated with anti-U1-nRNP antibody (correlation coefficient: 0.5, $P < 0.05$), anti-dsDNA antibody (correlation coefficient: 0.5, $P < 0.05$), anti-nucleosome antibody (correlation coefficient: 0.5, $P < 0.05$), and anti-histone antibody (correlation coefficient: 0.4, $P < 0.05$). The correlations between anti-$SmD1_{83-119}$ and anti-dsDNA, anti-U1-nRNP, anti-nucleosome, and anti-histone are shown in Figure 1. In brief, approximately 16 patients who were positive for anti-$SmD1_{83-119}$ were found to be simultaneously positive for anti-dsDNA, anti-U1-nRNP, anti-nucleosome, and anti-histone. While only 12 patients who were positive for anti-$SmD1_{83-119}$ were also positive for anti-Sm, indicating that anti-$SmD1_{83-119}$ antibody was more prevalent in cSLE than anti-Sm antibody. Further, these results show that anti-$SmD1_{83-119}$ antibody does not correlate with anti-SSA/Ro60 (correlation coefficient: 0, $P = 1.00$), anti-SSA/Ro52 (correlation coefficient: 0.12, $P = 0.43$), and anti-SSB antibodies (correlation coefficient: 0.18, $P = 0.24$).

Evaluating anti-SmD1-amino-acid 83-119 peptide autoantibody in children with other suspected autoimmune diseases

To further evaluate the clinical significance of anti-$SmD1_{83-119}$ autoantibody in laboratory diagnosis of cSLE, we performed additional analyses in children with non-SLE diseases. Sera from children with arthralgia ($n = 39$), hematuria ($n = 59$), or ITP ($n = 27$) were analyzed for anti-$SmD1_{83-119}$ antibody. As shown in Table 4, 12 of 39 (30.8%) sera samples from arthralgia patients, 24 of 59 (40.7%) sera samples from hematuria patients, and 9 of 27 (33.3%) sera samples from ITP patients were positive for anti-$SmD1_{83-119}$ antibody. Furthermore, one patient with arthralgia simultaneously exhibited anti-$SmD1_{83-119}$, anti-Sm, anti-dsDNA, and anti-nucleosome reactivities. Meanwhile, four samples from hematuria patients were positive for anti-$SmD1_{83-119}$ and anti-dsDNA and three samples were

Table 1: Prevalence of clinically relevant autoantibody species in patients with cSLE and other diseases

Patients	*N*	Anti-U1-nRNP	Anti-$SmD1_{83-119}$	Anti-Sm	Anti-SSA/Ro60	Anti-SSA/Ro52	Anti-SSB	Anti-Scl70	Anti-dsDNA	Anti-Nucleosome	Anti-Histone
SLE, *n*	46	22	36	12	23	14	8	2	21	20	18
AS, *n*	11	0	3	0	0	0	1	0	0	0	0
HSP, *n*	60	0	10	0	1	0	1	0	1	0	1

SLE: Systemic lupus erythematosus; AS: Ankylosing spondylitis; HSP: Henoch-Schonlein purpura; dsDNA: Double-stranded DNA; cSLE: Children with systemic lupus erythematosus; SmD183-119: SmD1-amino-acid $_{83-119}$ peptide.

Table 2: Sensitivity and specificity of anti-$SmD1_{83-119}$ antibody detection using an immunoblotting assay in cSLE and non-cSLE patients

Diagnosis	Our study			Reported study[10]		
	N	Sensitivity (%)	Specificity (%)	*N*	Sensitivity (%)	Specificity (%)
cSLE	46	78.3	90.8	167	70	91.7
AS	11	27.3	73.9	-	-	-
HSP	60	16.7	69.3	-	-	-

-: Not applicable; SLE: Systemic lupus erythematosus; AS: Ankylosing spondylitis; HSP: Henoch-Schonlein purpura; cSLE: Children with systemic lupus erythematosus.

Table 3: Correlation between anti-$SmD1_{83-119}$ positivity and other auto-antibody species in 46 cSLE patients (correlation coefficient)

Items	Anti-U1-nRNP	Anti-Sm	Anti-SSA/Ro60	Anti-SSA/Ro52	Anti-SSB	Anti-dsDNA	Anti-Nucleosome	Anti-Histone
Anti-$SmD1_{83-119}$	0.505	0.313	0	0.12	0.175	0.483	0.462	0.442
P	<0.001	0.034	1	0.429	0.244	0.001	0.001	0.002

dsDNA: Double-stranded DNA; cSLE: Children with systemic lupus erythematosus.

Table 4: Positive number of anti-SmD1$_{83-119}$ in children with suspected autoimmune diseases

Items	*N*	Anti-SmD1$_{83-119}$	Anti-SmD1$_{83-119}$ positive sera		
			Anti-Sm	Anti-dsDNA	Anti-Nucleosome
Arthralgia, *n*	39	12	1	1	1
Hematuria, *n*	59	24	0	4	3
ITP, *n*	27	9	0	1	1

The data was presented by *n*. ITP: Idiopathic thrombocytopenia purpura; dsDNA: Double-stranded DNA.

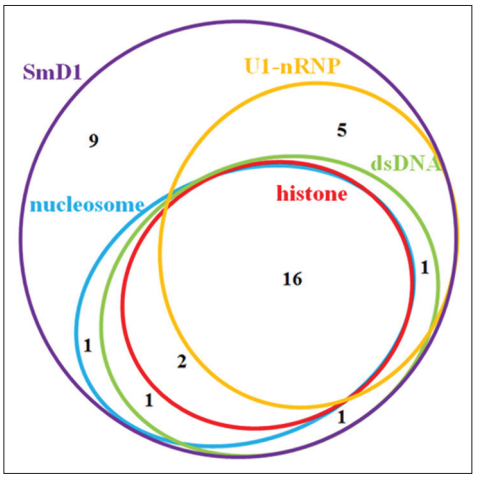

Figure 1: Correlation between anti-SmD1$_{83-119}$ positivity and other autoantibodies in cSLE patients. The number means the case number. dsDNA: Double-stranded DNA; SmD1$_{83-119}$: SmD1-amino-acid 83-119 peptide; cSLE: Children with systemic lupus erythematosus.

positive for anti-nucleosome antibody. One patient with ITP was positive for anti-SmD1$_{83-119}$ antibody, anti-dsDNA, and anti-nucleosome.

Discussion

SLE is a prototypic autoimmune disease with complex pathogenesis.[15,16] Adult SLE patients can produce a series of autoantibodies against nuclear and cytoplasmic antigens.[17,18] It is well known that anti-Sm autoantibody, which was first described by Tan and Kunkel,[1] is considered as a highly specific marker for adult SLE. Various techniques and different antigens have been used to detect anti-Sm antibody because anti-Sm antibody exhibits a low sensitivity (5–30%).[15,19] Further reports reveals that Sm protein is composed of at least nine different polypeptides termed according to their electrophoretic mobility on SDA-PAGE B (B1; 28,000), B' (B2; 29,000), N (B3; 29,500), D1 (16,000), D2 (16,500), D3 (18,000), E (12,000), F (11,000), and G (9000).[9,20] These proteins, but most frequently the SmB and SmD1 peptides, are anti-Sm antibody targets. As SmBB' and U1-nRNPs share the cross-reactive epitope motif, SmD1 is regarded as the most SLE-specific Sm antigen.[9,20] Through epitope mapping using 13-mer peptides with ten overlapping amino acids and sera from adult SLE patients, Riemekasten *et al*.[10] described that SmD1$_{83-119}$ could be recognized by 70% of adult SLE serum samples. Consistent with these results, 78.3% (36/46) of our cSLE serum samples exhibited reactivity against this peptide using an immunoblotting assay.

Previous studies reported that the SmD1$_{83-119}$ epitope is cryptic and not easy to be recognized by antibodies and this can partly explain the low sensitivity of anti-Sm antibody.[10,11] Investigators have also established that as the major protein component of milk, casein is likely an important cofactor for autoantibodies against the SmD1$_{83-19}$ peptide and functions through changing the peptide's critical epitope conformation.[7] Further, Dieker *et al*.[21] mentioned that binding between autoantibodies and SmD1$_{83-119}$ can be mediated by dsDNA and nucleosome. As shown in this study, anti-SmD1$_{83-119}$ reactivity positively correlated with anti-dsDNA, anti-histone, and anti-nucleosome antibodies. However, our findings could not exclude the possibility that binding was mediated by anti-dsDNA antibody or anti-nucleosome antibody; however, in one intriguing finding, 16 of the anti-SmD1$_{83-119}$-positive sera also reacted with dsDNA, nucleosome, and histone. This pattern may be used to generate high specificity (100%) compared with the SmD1$_{83-119}$ peptide alone and to generate better sensitivity (34.8%) compared with the Sm antigen (26.1%) in cSLE sera. These data suggest that co-detecting the four specific autoantibodies (SmD1$_{83-119}$, dsDNA, nucleosome, and histone as the antigens) may enhance specificity compared with only the anti-SmD1$_{83-119}$ antibodies or enhance sensitivity compared with only anti-Sm antibody detection in cSLE patients.

As anti-SmD1$_{83-119}$ antibody exhibited high sensitivity in the cSLE patients, we performed more experiments to evaluate the clinical significance in child diseases that share characteristics with SLE, such as arthralgia or hematuria. Our results suggest that frequency in the arthralgia patient group was similar to previous studies (12/39), while patients with hematuria showed a moderately higher frequency (24/59).[10] Intriguingly, one serum sample from an arthralgia child simultaneously showed responses to the SmD1$_{83-119}$ peptide, the Sm antigen, dsDNA, and nucleosomes. More experiments were performed which show that the antinuclear (ANA) antibody titer was 1:3200. A follow-up study further showed that the patient in that case was finally diagnosed with a mixed connective tissue disease, which is an autoimmune disease. In addition, serum samples from two hematuria patients exhibited reactivity to all the following antigens: SmD1$_{83-119}$ peptide, dsDNA, nucleosome, and histone. Further studies revealed that the ANA titer was 1:100, which is an indeterminate value indicating the weak association with autoimmune disorders. More studies are necessary to determine the pathogenesis.

In conclusion, the present study demonstrates that measuring sera reactivity with the SmD1$_{83-119}$ peptide in an immunoblotting assay may increase sensitivity in cSLE patients. These data further show a positive correlation between anti-SmD1$_{83-119}$ antibody and anti-dsDNA,

anti-nucleosome, and anti-histone antibodies. Combining the three autoantibodies with the anti-SmD1$_{83-119}$ antibody may enhance specificity for detecting cSLE.

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Conflicts of interest

There are no conflicts of interest.

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