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Autoantibodies against Cajal bodies in systemic lupus erythematosus

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Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: Cajal bodies (CB) are distinct sub-nuclear domains rich in small nuclear ribonucleoprotein particles (snRNPs); they are involved in pre-mRNA processing. Lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibody production against different nuclear molecules, including those involved in pre-mRNA processing. The aim of the present investigation is to assess the presence of anti-CB autoantibodies in a cohort of SLE sera.

Material/Methods: Antinuclear antibodies (ANA) were screened by indirect immunofluorescence in a batch of 190 sera from patients who met the ACR criteria for SLE classification; fine specificity was determined by Western blot using HEp-2 cells or rat hepatocyte extracts purified by ion exchange chromatography.

Results: Four sera had anti-Cajal body (CB) autoantibodies. Interestingly, all of these patients had intermittent extensive oral and esophageal ulceration. The autoantibodies to CB were of the IgG class, and by Western blot these sera had reactivity against an 80 kDa protein (coilin) associated with Sm proteins.

Conclusions: Anti-CB autoantibodies constitute an uncommon specificity of SLE; therefore it seems that anti-CB antibody specificity is associated with extensive mucous ulceration.

key words: **systemic lupus erythematous p80 coilin • small nuclear ribonucleoprotein particles (snRNPs) • pre-mRNA processing • autoantibodies • immunofluorescence**

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BACKGROUND

The Cajal body (CB) is a distinct sub-nuclear domain rich in small nuclear ribonucleoprotein particles (snRNPs), involved in pre-mRNA processing. Although the functions of CB are still under investigation, experimental data suggest that CBs are involved in the biogenesis, transport and recycling of small nuclear ribonucleoprotein particles (snRNPs). Nuclear structures representing CB contain an 80 kDa protein, coilin, whose function is not fully understood, although it forms complexes with U7, U1, U2, U4/U6 RNPs within CBs [1–3]. The microinjection of anti-coilin antibodies into living cells affects the structure of CBs [4], and is likewise affected by the injection of anti-(U1) snRNP autoantibodies. In addition, the homologous pairing activity of the human recombination complex is inhibited by the independent effect of both antibodies [5]. These results suggest a possible function of CB during the splicing of messenger RNA.

Andrade (1991) and Chan (1994) used a human autoantibody to clone and isolate the cDNA encoding p80 coilin [6,7]; however, no clear clinical association has been ascribed to anti-CB. Anecdotal reports have noted the presence of anti-coilin antibodies in inflammatory skin diseases, such as atopic dermatitis [8]. In a Japanese study, sera from 365 patients were analyzed and anti-coilin antibodies were found in eleven patients. None of these patients could be diagnosed with a differentiated rheumatic disease, except in two cases (one with systemic scleroderma and another with Sjögren's syndrome), but most of the patients with anti-coilin antibodies had general symptoms, including fatigue, arthralgia, headaches, dysmenorrhoea, lymph node swelling, and/or low grade fever, as well as chronic fatigue syndrome (CFS), and some of them showed low complement [9].

There has been no previous investigation of the prevalence and clinical relevance of anti-CB autoantibody in a cohort of systemic lupus erythematosus (SLE). Accordingly, the goal of the present investigation was to assess the presence of anti-CB autoantibodies in a cohort of SLE sera.

MATERIAL AND METHODS

One hundred and ninety sera from lupus patients who met the ACR criteria for SLE were screened by ANA test on HEp-2 cells [10]. Sera positive for CB were further characterized by Western blot. Sera specificity, such as anti-Sm and anti-RNP, was preformed by Ouchterlony using rabbit thymus extract (Immunovision, Inc. Springdale, Arizona, USA); anti-Ro and anti-La were determined by double diffusion in 1% agar using human spleen extract; anti-DNA was determined by immunofluorescence on *Crithidia luciliae*; anti-phospholipid antibodies were determined by ELISA (Binding Site). The clinical data of the patients with anti-CB autoantibodies are described below.

Human epithelial HEp-2 cells (ATTC, USA) were grown in DMEM (Gibco/BRL) containing 10% fetal calf serum and 100 units/ml of penicillin/streptomycin. Freshly isolated rat hepatocytes were grown in modified Waymouth medium [11]. The cells were suspended and plated in polystyrene flasks and cover slips at a density of 5×10^6/ml and incubated at 37°C in 5% CO_2 and 85% relative humidity, and used for antigen extraction or for immunofluorescence assays.

For antigen extraction, HEp-2 cells or rat hepatocytes were harvested and spun pelleted at 1200 rpm for 10 minutes, then resuspended in denaturing lysis buffer (1% SDS, 0.25M EDTA, 2mM bis-mercaptoethanol, 1 mM tris pH 7.5, 5mM PMSF, adjusted by H_2O to to 1 ml). The soluble antigens were extracted by ultrasonic disruption 5 times for 30 seconds by pulse, with an efficiency of 50%. The lysates were then centrifuged at 12,000 rpm for 10 minutes; the supernatants were saved and used for analysis. The protein concentration was measured using UV light by coefficient extinction at 280 nm.

For purposes of ion exchange chromatography, coilin was partially purified in a 2×50 cm column containing DEAE cellulose (DE52, Whartman, USA). Pre-swollen DEAE was equilibrated in 0.5M NaCl, 0.025M Tris, pH 7.5 (running buffer). HEp-2 cell extracts or rat hepatocyte extracts were dialysed against distilled water and lyophilized; the antigenic powder was resuspended in 50 ml of running buffer, then applied into the DEAE column and fractionated. The elution volume was 10 ml/tube, the amount of proteins was determined at 280 nm. Individual fractions were used to determine the active peak, which was used for SDS-PAGE and Western blot for sera testing. A monoclonal anti-coilin antibody (BD Biosciences Pharmingen, San Diego, California, USA) was used as control.

Total or purified cell extracts were submitted to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); the procedure was performed under reducing conditions, as described by Laemmli and Favre, in 11% polyacrylamide gels [12]. The unstained gels were blotted onto nitro-cellulose membranes, using the procedure described by Towbin [13]. Active sites were blocked with 1% non-fat milk. The blotted proteins were probed at room temperature for 1 hour with SLE sera containing anti-CB demonstrated by immunofluorescence. Bound antibodies were disclosed with rabbit anti-human IgG, IgA or IgM peroxidase conjugated antibodies (Sigma, St Louis, Missouri, USA). Immunoreactive bands were visualized with the ECL chemiluminescent kit (Amersham Pharmacia Biotech). Autoradiography was then performed using a Biomax X-ray film (Kodak, Rochester, New York, USA) and cassettes with intensifying screens.

The coiled bodies were examined on HEp-2 cells or in hepatocyte monolayers by indirect immunofluorescence. The SLE sera were incubated for 30 minutes on the cell monolayers, followed by three PBS washings, and then 30 minutes incubation was carried out with FITC-labeled monoclonal anti-human IgG, anti-IgA, or IgM (Sigma, St. Louis, Missouri, USA). After further washing the cover slips were mounted and the slides were evaluated by microscopy equipped with epifluorescence.

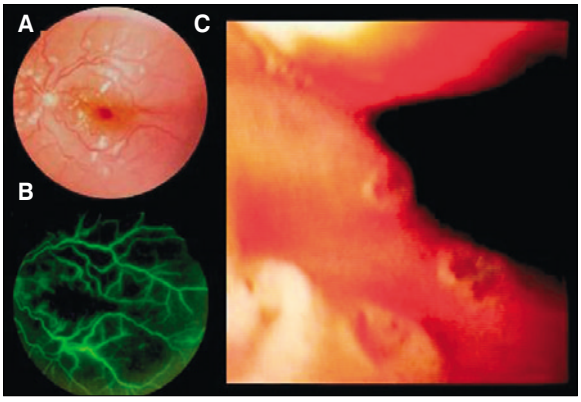

Figure 1. (**A**) Retineal vasculopathy in patient number 1; fundoscopy demonstrates multiple exudates. (**B**) Fluorangiography of patient 1, demonstrating that exudates are coincident with areas of retinal vasculitis. (**C**) Endoscopic image of esophageal ulcerations seen in patient number 3.

RESULTS

Four patients were found to have CB autoantiboides; they are described below.

Patient 1

This was an 18-year-old male, with acute disseminated rash triggered by sunlight exposure. The skin lesions were broadly distributed in face, torso and upper extremities, accompanied by palmar vasculitis. Additionally, he presented with extensive oral and esophageal ulceration, demonstrated by endoscopy, and arthritis. Six months later he had a relapse with retinal vasculitis (Figure 1A,B); at this time the ANA was positive, with a homogeneous pattern at a dilution of 1:10,240. This serum was also reactive to CB; additionally, the patient had positive anti-DNA (1:1280) and anti-Sm antibodies. The C3 and C4 fractions were decreased; moreover, the anti-phospholipid antibodies were positive by ELISA, and a skin biopsy demonstrated a positive lupus band test. Methyl prednisolone (1 gr) and cyclophosphamide 0.5 g/m^2 in bolus were administered monthly over a 6-month period. Additionally, he received 60 mg of prednisone daily. One month later, he developed acute transverse myelitis simultaneously with viral hepatitis (A type), so the antibodies against hepatitis virus A were positive with IgM and IgG isotypes. The patient improved steadily, with normalization of functional hepatic tests. After six months, total remission was observed, except for a residual plantar burning sensation secondary to transverse myelitis.

Patient 2

This 23-yar-old female had SLE which had evolved over three years. The disease was characterized by mucocutaneous involvement, including butterfly rash, palmar vasculitis, Raynaud phenomenon, extensive oral and esophageal ulceration. She also had transitory mental confusion, isolated seizures, nausea and vomiting. The CSF was aseptic, and the CAT scan was normal. She had positive ANA (1:2560) with reactivity to CB and anti-Sm autoantibodies. Intravenous cyclophosphamide and methylprednisolone bolus were administred monthly over a 6-month period. She went into remission, which was maintained with 30 mg of prednisone. Six months later, no residual neurological damage was demonstrated by clinical examination, CAT scan, and EEG studies.

Patient 3

This 24-year-old female had a disease that evolved over six months and was characterized by extensive oral, esophageal (endoscopy) and anal ulceration (Figure 1C). She also had intermittent fever, Raynaud's phenomenon, and alopecia. Her serology demonstrated a positive ANA (1:640) with speckled pattern and also reactivity with CB. An unexposed skin biopsy exhibited a positive lupus band test. Prednisone (20 mg daily) was administered and the ulcerations disappeared.

Patient 4

This 52-year-old female developed fever, extensive oral ulceration, arthritis, photosensitivity, malar rash, and Raynaud phenomenon over four years of clinical observation. She had positive speckled pattern ANA (1:1280), with some speckles that corresponded to CB. Anti-Sm antibodies were also detected. She was treated with 30 mg of prednisone daily.

Purified chromatographic fractions

Total cell extracts were partially purified by ion exchange chromatography; individual fractions were collected, dialyzed and lyophilised. The active fraction was eluted in the second peak (tube number 13); this fraction reacted with the monoclonal anti-coilin antibody as well as the SLE sera containing antibodies anti-CBs (Figure 2).

Sera from patients with anti-CB antibodies demonstrated by immunofluorescence exhibited simultaneous anti-Sm reactivity (i.e. proteins B and B' and D) as demonstrated by Western blot. In addition, these sera recognized a non-Sm ~80 kDa protein. Similar assays were carried out with the ion exchange purified fraction, and an ~80 kDa protein (coilin) in the active peak was specifically recognized by the monoclonal anti-coilin antibody, as well as by anti-CB sera. This autoantibody was further characterized as follows: A preparative SDS-PAGE was pre-formed using the active peak, which was electro-transferred onto a nitrocellulose sheet; the 80 kDa band was dissected and incubated 12 hours with a 1:50 dilution of anti-CB serum. The nitrocellulose strips were then extensively washed; bound antibodies were eluted with a 0.2M, pH 2.8 glycine buffer, and then neutralized with 0.1M tris, pH 8.9. Eluted antibodies were tested by indirect immunofluorescence on HEp-2 cells and by Western blot. Affinity-purified antibodies specifically recognized the Cajal bodies by immunofluorescence; they also recognized a unique 80 kDa protein which corresponded to coilin. All anti-CB sera were of the IgG class as determined by the isotype specific reagents described in the Methods section (Figure 2).

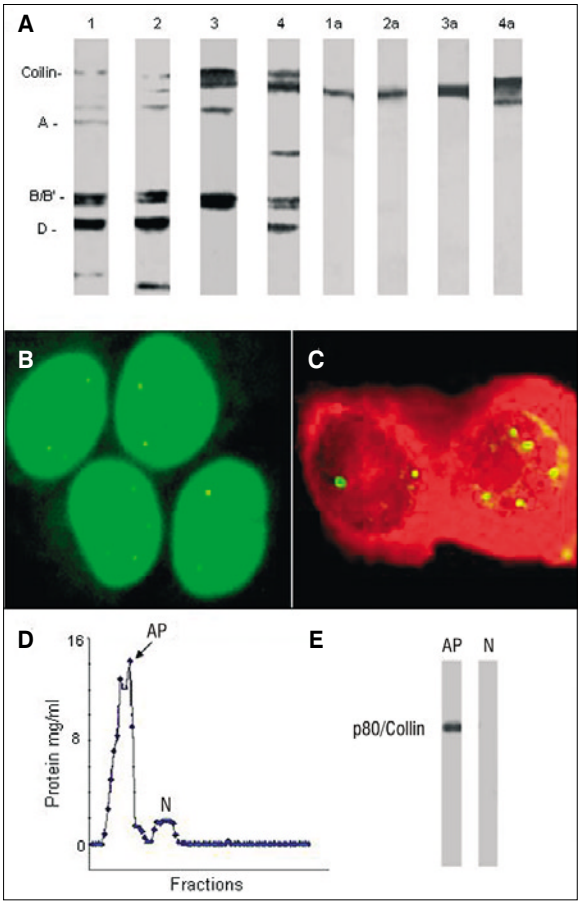

Figure 2. (**A**) *Western blot* showing the individual SLE sera reactivity against total HEp-2 cell extracts (lines 1 to 4). The sera recognized several Sm peptides (B and B' and D) and a non-Sm ~80 kDa protein. Autoantibodies (lines 1a–4a) reacted with the DEAE active peak; they recognize an ~80 kDa protein. (**B**) Indirect immunofluorescence on HEp-2 cells, showing a positive ANA, with speckled pattern and a few prominent dots corresponding to CB. (**C**) Affinity-purified antibodies eluted from the 80 kDa band tested by indirect immunofluorescence on HEp-2 cells recognize exclusively CBs (contrasted by Evans blue). (**D**) Ion exchange purification in DEAE cellulose. The chromatogram shows the active peak indicated by an arrow (AP), tube 13, and a shoulder (N) used as negative control. (**E**) Monoclonal anti-coilin antibody recognized the AP fraction, and was negative with the N fraction. AP was specifically recognized by sera with anti-CB antibodies, as demonstrated by Western blot in lines 1a–4a.

Discussion

The present study found that the frequency of autoantibodies against CBs in a cohort of SLE sera was 4 out of 190 (~2%). Although with such a frequency anti-CB could be considered rare or uncommon, this is not unlike the frequency of autoantibodies to other well-known autoantigens, such as proliferating cell nuclear antigen (PCNA) [14].

A common clinical feature shared by these patients included extensive mucous ulceration; in two patients the ulceration was documented by endoscopy.

The frequency of autoantibodies against CB in SLE has not previously been reported. Anti-CB may frequently be masked by other anti-nuclear antibodies, making the identification of CBs a difficult task. In addition, sera may have antibodies that produce similar nuclear dot staining with reactivity to gems, PML bodies, SP100 and other nuclear components [15–17].

In the present study, four SLE sera reactive to CB were identified, and these sera also recognized various Sm proteins as well as coilin. The specificity of these antibodies was demonstrated when the antibodies affinity-purified from the 80 kDa band reacted with CBs by immunofluorescence.

At present it is difficult to specify the clinical significance of anti-CB. However the extensive mucosal ulceration in these patients proved to be an interesting clinical observation. It is difficult to confirm that anti-CB is a marker of esophageal ulceration in SLE, since mucous involvement is a common feature and endoscopy is not usually performed. The presence of occlusive retinopathy in patient no. 1 can probably be attributed to an anti-phospholipid syndrome rather than to anti-CB autoantibodies [18].

From the immunochemical point of view, the anti-CB autoantibodies were restricted to IgG class, and this is a strong evidence that they are the product of a secondary immune response. Interestingly, this response is apparently linked to an anti-Sm immune response as well. This notion is supported by the observation that coilin complexes with U1, U2, snRNPs and Sm proteins within the CB [19]. In addition, it would appear that this linked response is driven by true antigens rather than exogenous agents [20].

Conclusions

Autoantibodies against CB constitute a rare specificity in SLE, and is clustered with anti-Sm autoantibodies. Anti-CB are clinically associated with extensive mucous ulceration.

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References:

1. Gall JG: Cajal Bodies: The first 100 years. Annu Rev Cell Dev Biol, 2000; 16: 273-300
2. Frey MR, Matera AG: Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. Proc Natl Acad Sci USA, 1995; 92: 5915-19
3. Carmo-Fonseca M, Pepperkok R, Carvalho MT, Lamond AI: Transcription-dependent colocalization of the U1, U2, U4/U6, and U5 snRNPs in coiled bodies. J Cell Biol, 1992; 117: 1-14
4. Almeida F, Saffrich R, Ansorge W, Carmo-Fonseca M: Microinjection of anti-coilin antibodies affects the structure of coiled bodies. J Cell Biol, 1998; 142: 899-912
5. Dowjat K: Anti-(U1)snRNP autoantibodies inhibit homologous pairing activity of the human recombination complex DNA. Cell Biol, 1997; 16: 819-27

6. Andrade LE, Chan EK, Raska I et al: Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. J Exp Med, 1991; 173: 1407-19
7. Chan EKL, Takano S, Andrade LEC et al: Structure, expression and chromosomal localization of human p80-coilin gene. Nucleic Acids Res, 1994; 22: 4462-69
8. Muro Y: Autoantibodies in atopic dermatitis. J Dermatol Sci, 2001; 25: 171-78
9. Onouchi H, Muro Y, Tomita Y: Clinical features and IgG subclass distribution of anti-p80 coilin antibodies. J Autoimmun, 1999; 13: 225-32
10. Tan EM, Cohen AS, Fries JF et al: The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 1982; 25: 1271-77
11. Zhang BH, Horsfield BP, Farrell GC: Chronic ethanol administration to rats decreases receptor-operated mobilization of intracellular ionic calcium in cultured hepatocytes and inhibits 1, 4, 5-inositol trisphosphate production: Relevance to impaired liver regeneration. J Clin Invest, 1996; 98: 1237-44
12. Laemmli UK, Favre M: Maturation of the head of bacteriophage T4 I DNA packaging events. J Mol Biol, 1973: 80: 575-99
13. Towbin HT, Staehlin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA, 1977; 76: 4350-54
14. Von Muhlen CA, Tan EM: Autoantibodies in the diagnosis of systemic rheumatic diseases. Semin Arthritis Rheum, 1995; 24: 323-58
15. Matera G: Of coiled bodies, gems, and salmon. J Cell Biochem, 1998; 70: 181-92
16. Zuchner D, Sternsdorf T, Szostecki C et al: Prevalence, kinetics, and therapeutic modulation of autoantibodies against Sp 100 and promyelocytic leukemia protein in a large cohort of patients with biliary cirrosis. Hepatology, 1997; 26: 1123-30
17. Matera AG: Nuclear bodies: multifaceted subdomains of the chromatine space. Trends Cell Biol, 1999; 9: 302-9
18. Jonas J, Kolbe K, Volcker HE, Kalden JR: Central retinal artery occlusion in Sneddon's disease associated with antiphospholipid antibodies. Am J Ophtalmol, 1986; 102: 37-40
19. Spector DL, Fu X-D, Maniatis T: Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J, 1991; 10: 3467-81
20. Tan EM, Chan EK, Sullivan KF, Rubin RL: Short analytical review: Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. Clin Immunol Immunopathol, 1988; 47: 121-41