Anti-U5 snRNP antibody as a possible serological marker for scleroderma–polymyositis overlap

M. Kubo, H. Ihn, M. Kuwana1, Y. Asano, T. Tamaki, K. Yamane and K. Tamaki

Department of Dermatology, Faculty of Medicine, University of Tokyo and 1Institute for Advanced Medical Research, School of Medicine, Keio University, Tokyo, Japan

Abstract

Objective. To determine the prevalence of the anti-U5 small nuclear ribonucleoprotein (snRNP) antibody in patients with systemic sclerosis.

Methods. Sera from 281 patients with systemic sclerosis, including 10 patients with overlapping polymyositis, were assayed using RNA immunoprecipitation and protein immunoprecipitation.

Results. Only one serum sample showed precipitation of U5 snRNA with scarce precipitation of U2, U1, U4 and U6 snRNAs. In addition, the serum precipitated a 200 kDa protein. The serum was from a 35-yr-old Japanese male patient with overlapping systemic sclerosis and polymyositis accompanied by large-cell lung carcinoma. The clinical appearance was similar to that of a case reported previously.

Conclusion. The presence of the anti-U5 snRNP antibody in serum may be specific for scleroderma–polymyositis overlap syndrome.

KEY WORDS: Scleroderma–polymyositis overlap, Anti-U5 snRNP antibody, Lung carcinoma, Immunoprecipitation.

A high prevalence of autoantibodies in the serum is one of the characteristics of collagen diseases. Previous studies have revealed many disease-specific autoantibodies and non-disease specific autoantibodies for each connective tissue disease, including systemic sclerosis (SSc) and polymyositis/dermatomyositis (PM/DM). The anti-topoisomerase I antibody, anti-centromere antibody, anti-Th/T0 antibody and anti-U3 small nuclear ribonucleoprotein (snRNP) antibody have been reported to be specific for SSc [1]. Several antisynthetase antibodies are well known in PM/DM; these include the antibodies to histidyl transfer ribonucleic acid (tRNA) synthetase (anti-Jo-1), threonyl-tRNA synthetase (anti-PL-7), alanyl-tRNA synthetase (anti-PL-12), isoleucyl-tRNA synthetase (anti-OJ) and glycyl-tRNA synthetase (anti-EJ) [2]. Furthermore, antibodies to signal recognition particles (SRP) are also known to be autoantibodies that are specific to PM/DM [3].

Overlapping SSc and PM is one of the most common and heterogeneous overlap syndromes. Several specific autoantibodies in SSc–PM overlap syndrome have been reported. The anti-PM-Scl antibody in Caucasian patients and anti-Ku antibody in Japanese patients are well-known disease-specific autoantibodies [3, 4]. In addition, anti-U1 snRNP antibody is known as one of the autoantibodies that show relatively low specificity for SSc, PM or SSc–PM overlap syndrome.

The anti-U5 snRNP antibody without coexisting antibodies to other snRNPs has been found previously in only one patient (LaJ) [5]. The serum was obtained in a survey of 1171 patients with collagen diseases, of whom 164 had the overlap syndrome [5]. Therefore, the existence of the anti-U5 snRNP antibody without coexisting anti-Sm antibodies or anti-U1 RNP antibodies is rare. Patient LaJ was diagnosed as having SSc with muscular involvement [5]. Accumulation of data may establish the clinical appearance of patients with the anti-U5 snRNP antibody. Therefore, we surveyed sera from Japanese patients with SSc to determine the prevalence of the anti-U5 snRNP antibody in such patients.

Materials and methods

Patients and serum samples

We studied 281 serum samples from Japanese patients with SSc (n = 271) and SSc–PM overlap syndrome.
who had visited our hospital during the last 15 yr. Disease classification was based on published criteria for SSc [6] and PM/DM [7]. Each sample was obtained at the first patient visit and stored at −20 °C or lower. Patients were not selected.

Reference antibodies
The reference antibodies were sera from patients with connective tissue diseases and were positive for anti-U1 snRNP, anti-Sm and anti-U5 snRNP (LaJ serum) antibodies [5]. Normal human serum was obtained from healthy volunteers.

Immunoprecipitation
We identified antinuclear antibodies by the use of a slight modification of a method described by Forman et al. [8] for protein A agarose assisted RNA immunoprecipitation using unlabelled cell extracts. Briefly, protein A agarose (Gibco BRL, Gaithersburg, MD, USA) was reacted with serum for 12 h at 4 °C and incubated with extracts of HeLa cells for 2 h at 4 °C. After washing three times with an NET-II buffer, the RNA fragment was precipitated using phenol–chloroform–isoamyl alcohol. The RNA was blotted with 10% urea polyacrylamide gel. The blotted RNA was detected with a Silver-stain Plus kit (Bio-Rad, NY, USA). Immunoprecipitation assays for protein analysis were also performed using [35S]methionine-labelled HeLa cell extracts.

Other immunological methods
Indirect immunofluorescence studies with human laryngeal tumour (HEp-2) cells as a substrate were performed as described elsewhere [9].

Results

Screening for serum antibodies against U snRNPs
All serum samples were tested in immunoprecipitation assays for nucleic acid analysis. Through this screening, we found one serum sample that immunoprecipitated U5 snRNP strongly and four weak nucleic acid bands with electrophoretic mobility identical to U2, U1, U4 and U6 snRNAs respectively (Fig. 1, lane 5). We named the serum SaT. Its profile was quite similar to that of LaJ serum (Fig. 1, lane 4) and clearly distinct from that of anti-Sm antibodies (Fig. 1, lane 3). The band for U5 snRNA was not found in samples from healthy controls (Fig. 1, lane 2).

In immunoprecipitation assays for protein analysis, a 200 kDa protein which was specific for U5 snRNP was found in anti-Sm serum, LaJ serum and SaT serum (Fig. 2, lanes 2, 4 and 5, respectively). The protein was not detected in sera from healthy controls (Fig. 2, lane 1) or in serum containing anti-U1 snRNP antibodies (Fig. 2, lane 3).

FIG. 1. RNA immunoprecipitation. Lane 1, total RNAs; lane 2, a normal control subject; lane 3, an SLE patient with the anti-Sm antibody; lane 4, patient LaJ, previously reported to have the anti-U5 snRNP antibody; lane 5, the patient in this case report (SaT). In lane 2, no immunoprecipitated snRNA was found and in lane 3 all the U1, U2, U4, U6 snRNAs were precipitated strongly. In lanes 4 and 5, only U5 snRNP was precipitated strongly and U1, U2, U4, U6 snRNA were precipitated weakly.

FIG. 2. Protein precipitation. Lane 1, a normal control subject; lane 2, an SLE patient with anti-Sm antibodies; lane 3, an SSc patient with anti-U1 snRNP antibodies; lane 4, patient LaJ; lane 5, the patient in this case report (SaT). A 200 kDa protein was precipitated in lanes 2, 4 and 5 but not in lanes 1 and 3.
Clinical features of patient SaT

The index patient, SaT, was a 35-yr-old Japanese man who visited our hospital with diffuse pigmentation over his whole body for 7 months; he had stiffness in his fingers, which he had noticed 6 months earlier, and had had proximal muscle weakness for 1 month. He lost 15 kg in weight over 6 months. Diffuse skin sclerosis and pigmentation over his whole body, nailfold bleeding and dysphasia were also noticed at his first visit. No cutaneous eruptions suggesting dermatomyositis, including heliotropic coloration, Gottron’s papules and itchy scratch dermatitis on the trunk, were noted.

Laboratory examination revealed marked elevations in creatine kinase activity (2888 U/l, normal range 44–166 U/l), serum aldolase activity (49.0 U/ml, normal range 1.7–5.7 U/ml) and lactate dehydrogenase activity (934 U/l, normal range 125–237 U/l). Indirect immunofluorescence studies using HEp-2 cells as the substrate demonstrated coarse speckled nuclear staining and fine reticular cytoplasmic staining (Fig. 3). Anti-U1 snRNP antibody, anti-Sm antibody, anti-topoisomerase I antibody, anti-Jo-1 antibody, anti-Ku antibody, anti-Mi-2 antibody, anti-SS-A/Ro antibody and anti-SS-B/La antibody were not found in the serum in double immunodiffusion tests. Chest examination by radiography and computed tomography showed coin-like shadows in the hilums on both sides, with no evidence of interstitial pneumonia. Electromyography showed a myogenic pattern. No swelling of the superficial lymph nodes was seen and no abnormal mass, except for the lung tumour, was found on examination by abdominal computed tomography and abdominal ultrasonography. Electromyography disclosed atroventricular blocks of the Wenckebach type.

A skin biopsy specimen from the left forearm revealed dense basal pigmentation, enlargement of collagen bundles and an increase in their number, and perivascular infiltration of inflammatory cells. No evidence of dermatomyositis, including the deposition of mucinous material or liquefactive degeneration, was found.

A muscle biopsy specimen from the left quadriceps showed necrosis of the muscle cells and infiltration of inflammatory cells between the muscle cells. Transbronchial lung biopsy disclosed large, irregular tumour cells.

From the clinical and histopathological findings and laboratory data, we diagnosed this patient as having PM–scleroderma overlap syndrome accompanied by lung cancer. He was treated with three courses of chemotherapy with cisplatin and etoposide. The coin lesions in the chest disappeared and the skin sclerosis improved moderately, but with no improvement in the myositis. No recurrence of lung cancer was found after 2 yr with local radiation treatment. The activities of myogenic enzymes were eventually reduced to normal ranges after treatment with a combination of systemic corticosteroid, systemic azathioprine and systemic gammaglobulin. No improvement was seen in his cardiac conduction defect. He was still positive for anti-U5 snRNP antibody after treatment.

Discussion

The U snRNPs are RNA–protein complexes of small nuclear RNA that are rich in uridine and several proteins, and exist in the nucleus or nucleoplasm [10]. U5 snRNP exists in the nucleoplasm and includes Sm core proteins and a doublet of a specific 200 kDa protein [11]. U5 snRNP is dedicated to the splicing of heterogeneous nuclear RNA to messenger RNA (mRNA) with U1, U2, U4, U6 snRNP, including Sm core proteins.

An antibody to U5 snRNP not coexisting with anti-Sm antibodies has been found previously in only one patient, who was also diagnosed as having an overlap syndrome of SSc and myositis, among 1171 patients with various connective tissue diseases [5]. We examined our stock of sera from 281 patients with SSc, including 10 patients with SSc–PM overlap syndrome, for the presence of specific antinuclear antibodies. However, the presence of the anti-U5 snRNP antibody without the coexistence of the anti-Sm antibody or anti-U1 snRNP antibody was not found in the stock sera except for serum from one patient. This patient showed clinical symptoms similar to those of the patient with the anti-U5 snRNP antibody reported previously [5]. Both male patients showed SSc and PM without lung fibrosis. Furthermore, they both showed cardiac conduction defect. However, our patient showed diffuse cutaneous SSc and lung carcinoma, while the previous patient showed only limited cutaneous SSc. Thus, the antibodies to U5 snRNP without anti-Sm antibodies may be a marker for SSc–PM syndrome.

In conclusion, the results of this study, and those of the previous report [5], suggest that the anti-U5 snRNP antibody is rare, but that it may be a specific marker for overlapping SSc and PM.
Acknowledgement

We wish to thank Drs Yutaka Okano and Thomas A. Medsger for providing the LaJ serum used in this report.

References