0022-1767/86/13712-3802$02.00/0
THE JOURNAL OF IMMUNOLOGY
Vol. 137, 3802–3808, No. 12, December 15, 1986
Printed in U.S.A.

IMMUNOLOCALIZATION AND PARTIAL CHARACTERIZATION OF A NUCLEOLAR AUTOANTIGEN (PM-Scl) ASSOCIATED WITH POLYMYOSITIS/SCLERODERMA OVERLAP SYNDROMES[1]

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Precipitating anti-PM-Scl antibodies are present in sera from patients with polymyositis, scleroderma, and polymyositis/scleroderma overlap syndromes. By indirect immunofluorescence microscopy, anti-PM-Scl antibodies stained the nucleolus in cells of different tissues and species, suggesting that the antigen is highly conserved. By electron microscopy, anti-PM-Scl antibodies reacted primarily with the granular component of the nucleolus. Drugs that inhibit rRNA synthesis had a marked effect on the expression of PM-Scl antigen. In actinomycin D-treated cells, immunofluorescence staining by anti-PM-Scl was significantly reduced with residual staining restricted to the granular regions of nucleoli. Treatment with 5,6-dichloro-β-D-ribofuranosylbenzimidazole (DRB) also selectively reduced nucleolar staining. On a molecular level, anti-PM-Scl antibodies precipitated 11 polypeptides with molecular weights (M_r) ranging from 110,000 to 20,000. The M_r 80,000 and 20,000 polypeptides were phosphorylated. Evidence suggests that the PM-Scl antigen complex may be related to a preribosomal particle.

Autoantibodies against nucleic acids and a variety of cellular proteins and RNA protein complexes are produced by patients with systemic autoimmune disease (1). These serum antibodies not only are of diagnostic significance, e.g., anti-native DNA and anti-Sm antibodies for systemic lupus erythematosus (2, 3), but also have proved to be valuable immunologic tools in molecular and cellular biology. For example, antibodies against U1 ribonucleoprotein were shown to effectively inhibit splicing of heterogeneous nuclear RNA, thus implicating this nuclear ribonucleoprotein particle in the processing of mRNA (4). Most recently, antibodies against the Sm ribonucleoprotein particle that is complexed with uridine-rich small nuclear RNA, named U1, U2, U4, U5, and U6 (5), have facilitated cloning of cDNA that contains sequences complementary to mRNA for the E protein (M_r 11,000) of the Sm particle (6).

In a recent paper, a new precipitating antigen-antibody system termed PM-Scl was described in patients with polymyositis, scleroderma, and polymyositis/scleroderma overlap syndromes (7). These antibodies have identical specificities as anti-PM-1 antibodies, which had been previously reported in patients with polymyositis (8). The immunofluorescence patterns obtained with anti-PM-Scl sera were initially described as being both nucleolar and nucleoplasmic in nature. Recently, Targoff and Reichlin (9) provided evidence for a nucleolar localization of the PM-Scl antigen. Although some M_r data on the PM-Scl antigen were recently reported by Bernstein et al. (10), the macromolecular nature and origin of the PM-Scl antigen remain unknown. To understand the significance of autoantigens in the context of diseases with which they are associated, it may be important to elucidate their structure and function. In this study, we have shown that the PM-Scl antigen is a complex particle composed of at least 11 polypeptides. We further provide evidence that the PM-Scl antigen complex may be of preribosomal origin.

MATERIALS AND METHODS

Patient sera. Anti-PM-Scl sera were obtained from patients with polymyositis, scleroderma, or polymyositis/scleroderma overlap syndromes and were selected from the serum bank of this laboratory. Their immunologic specificity was assessed by double immunodiffusion against a reference anti-PM-Scl serum (S.B.) as described (7). This serum had also been the standard reference serum in the latter study.

Indirect immunofluorescence. Sera were diluted starting at 1/40 in phosphate-buffered saline (PBS), pH 7.4, and used for indirect immunofluorescence. Substrates included commercially available Hep-2 cells (Bion, Park Ridge, IL) and KB cells (Electronucleonics, Bethesda, MD). Other substrates were Vero and HeLa cells grown on microscopic slides and fixed in acetone/methanol at 3:1 for 3 min, unstimulated and concanavalin A (Con A)-stimulated human peripheral blood lymphocytes, T cell lymphoma Molt 4 cells, rat RV cells, rat kangaroo PTK2 cells, fish FHM cells, turtle TH-1 cells, duck (Pekin duck) embryonic cells, and rat liver cryostat sections. The duck embryonic, FHM, and TH-1 cells were obtained from the American Type Culture Collection, Rockville, MD; the other cells, from stocks in this and Dr. Scheer's laboratory. A fluorescein goat anti-human IgG conjugate (Tago, Burlingame, CA) was the detecting reagent. Intensity of immunofluorescence was graded from weak (+), to moderate (++), to strong (+++). Double immunofluorescence staining with the use of a mouse monoclonal antinucleolar antibody of IgG2a isotype and human anti-PM-Scl serum was performed as

Received for publication May 24, 1986.
Accepted for publication September 2, 1986.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This is Publication No. 4340BCR from the Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, CA. This work was supported by National Institutes of Health Grants AM32063 and AI10386. G. Reimer was supported by Grant Re 585/1-1 from the Deutsche Forschungsgemeinschaft.
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described (11). Results were read on a Leitz Ortholux II fluorescence microscope.

Electron microscopic immunocytochemistry. A representative anti-PM-Scl serum (S125) was diluted 1/100 in PBS and incubated for 2 hr with frozen sections (5-μm thickness) of regenerating rat liver that had been fixed in acetone at −20°C for 10 min. After several washes in PBS, goat anti-human IgG coupled to colloidal gold particles of 5-nm diameter (Janssen Life Sciences, Beerse, Belgium) was added at a dilution of 1/4. After overnight incubation at 4°C, the specimens were fixed and processed for electron microscopy as described in detail elsewhere (12).

Drug studies. Tissue culture Vero cells were incubated with medium containing 0.1 μg/ml actinomycin D for 4 hr and with 50 μg/ml 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB),[4] a halogenated adenosine analogue, for 6 hr. Both drugs were purchased from Behring Diagnostics, La Jolla, CA. After drug treatment, cells were fixed and processed for indirect immunofluorescence or double immunofluorescence staining as described.

Nuclease digestion experiments. For nuclease digestion experiments, Vero cells were grown on microscopic slides and fixed as described above. The cells were then digested with 0.25 mg/ml DNase I (Millipore, Freehold, NJ) in PBS containing 5 mM $MgCl_2$ and 2 mM phenylmethylsulfonylfluoride (PMSF) or with 0.25 mg/ml RNase A (Millipore) in PBS for 30 min at 37°C. After digestion, cells were washed in PBS and processed for indirect immunofluorescence. Buffers without enzymes served as negative controls.

Immunoprecipitation. HeLa cells were metabolically labeled with [^{35}S]methionine and [^{32}P]orthophosphate (New England Nuclear, Boston, MA), lysed in buffer A (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM $MgCl_2$, 0.5% Nonidet P-40, and 2 mM PMSF), and further processed as described (13) with modifications. All experiments were performed in Eppendorf microcentrifuge tubes at 0°C to 4°C. In brief, samples of 10 μl of patient serum were mixed for 1 hr with 100 μl of protein A-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) suspended at 10% (v/v) in buffer B (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM EDTA, 0.1% deoxycholate, 0.1% sodium dodecyl sulfate [SDS] 0.5% Nonidet P-40 23 U/ml Kallikrein inactivator [Behring Diagnostics]) supplemented with 2 mg/ml bovine serum albumin. After washing with buffer B, 100 μg of ^{35}S- or ^{32}P-labeled HeLa cell extract was added and incubated for 1 hr with constant mixing. The beads were then washed five times in buffer B, boiled in Laemmli sample buffer (14) and processed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography at −70°C. Half of the ^{32}P pellet was processed by SDS-PAGE and the other half was phenol extracted; any RNA was precipitated in ethanol. RNA analysis was performed in 7 M urea, 8% polyacrylamide gels followed by autoradiography as described (5).

RESULTS

Immunofluorescence staining with anti-PM-Scl antibodies with the use of different cell substrates. Various cell lines and tissues were used as substrates for staining with anti-PM-Scl sera from different patients. The staining patterns observed with a representative anti-PM-Scl serum (S125) are shown in Figure 1. In human HeLa cells (Fig. 1*a* and *b*), monkey Vero cells (Fig. 1*c* and *d*), and mitogen-stimulated human peripheral blood lymphocytes (Fig. 1*e* and *f*), anti-PM-Scl antibodies strongly (+++) stained the interior of nucleoli. Weak nucleoplasmic staining (+) was also observed with most of these substrates. The homogeneous nucleolar staining pattern was uniformly observed in all substrates tested, including cells such as human Molt 4 cells, rat RV cells, marsupial PtK2 cells, and rat liver sections. In unstimulated peripheral blood lymphocytes, PM-Scl staining was considerably weaker (+) when compared with Con A-stimulated lymphocytes, which gave a (+++) staining (Fig. 1*e* and *f*). In addition, Pm-Scl staining was also present in fish FHM cells, turtle TH-1 cells, and a duck embryo cell line, suggesting that the epitopes recognized by anti-PM-Scl antibodies are widely conserved in evolution. All anti-PM-Scl sera tested so far produced comparable nucleolar staining patterns in different cell substrates.

[4] Abbreviations used in this paper: PMSF, phenylmethylsulfonylfluoride; DRB, 5,6 dichloro-β-D-ribofuranosylbenzimidazole.

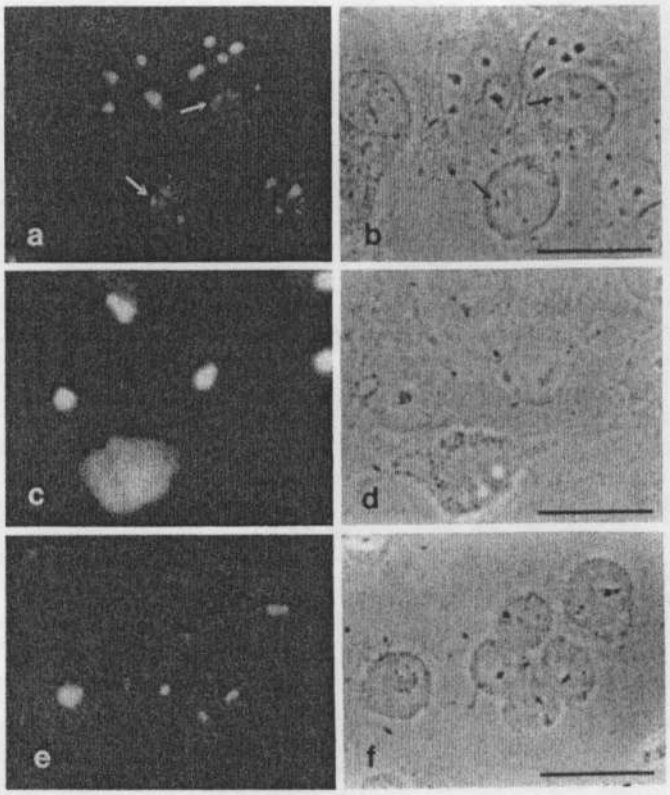

Figure 1. Immunofluorescence staining of HeLa cells (*a*), Vero cells (*c*), and Con A-stimulated human peripheral blood lymphocytes (*e*) with autoimmune human anti-PM-Scl antibodies (S125). All nucleoli demonstrate bright homogeneous nucleolar staining in all cells tested. Weak nucleoplasmic staining is also present, especially in Vero cells. Note prenucleolar body staining (*arrows*) of a telophase HeLa cell in *a*. Similar results were obtained with all the sera tested. For comparison, the cells are shown in phase contrast (*b*, *d*, and *f*). Bars 10 μm, ×1450.

Anti-PM-Scl staining during cell cycle. Figure 2*a* and *b* shows indirect immunofluorescence with rat kangaroo PtK2 cells used as substrate when reacted with a representative anti-PM-Scl serum (S125) from a patient with scleroderma. In interphase cells, immunofluorescence staining was predominantly within the nucleolus with weak (+) staining of the nucleoplasm. In metaphase cells, diffuse staining was observed in the area of the condensed chromosomes. In anaphase cells, anti-PM-Scl staining was diffusely distributed around the chromosomes and in the perichromosomal cell plasm. In telophase, anti-PM-Scl antibodies stained the prenucleolar bodies of the reconstituting nucleoli, as shown for HeLa cells in Figure 1*a* (*arrows*).

Subnucleolar localization of PM-Scl by electron microscopic immunocytochemistry. For subnucleolar localization of the reactive antigen, electron microscopic immunocytochemistry was performed with the immunogold label technique. Figure 3*a*, *b*, and *c* shows sections through nucleoli of regenerating rat liver. IgG from a representative anti-PM-Scl serum (S125) was localized primarily in the granular component of nucleoli. Gold-antibody complexes were selectively enriched over the granular component with accentuation over the periphery of the nucleoli. Immunogold was virtually absent in the dense fibrillar component or fibrillar centers. It should be noted that gold-antibody complexes were also observed in distinct clusters dispersed throughout the nucleoplasm as pointed out by arrows (Fig. 3*a* and *c*).

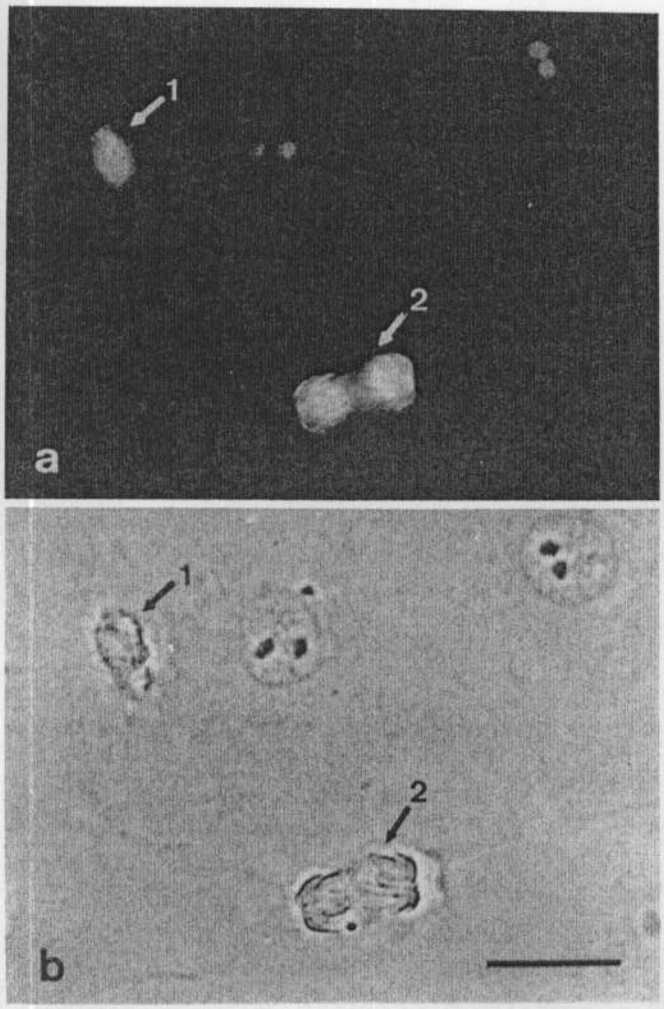

Figure 2. Immunolocalization of anti-PM-Scl antibodies (S125) in rat kangaroo PtK2 cells during different phases of the cell cycle, as visualized by immunofluorescence microscopy (*a*) and phase contrast (*b*). In interphase cells, bright nucleolar staining and weak nucleoplasmic staining by anti-PM-Scl is present. Note that nucleolar staining relocates as the chromosomes condense during metaphase (*arrow 1*) giving rise to diffuse staining in the area of the condensed chromosomes. In late anaphase cell (*arrow 2*), PM-Scl staining is diffusely distributed on the surface of the chromosomes and in the perichromosomal cell plasm. Individual chromosomes can be distinguished in phase-contrast optics (*b*) as elongated lighter structures surrounded by dark borders. Bar 20 μm. ×1040.

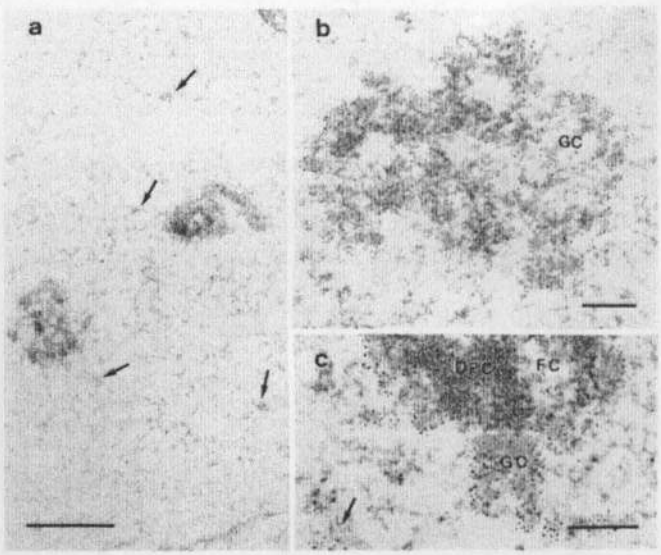

Figure 3. Immunolocalization of anti-PM-Scl antibodies (S125) in subnucleolar compartments in the hepatocytes of regenerating rat liver, as shown by the electron microscopic immunogold label technique. For orientation, frame *a* of this composite shows sections of two nucleoli and surrounding heterochromatin at low magnification. Frames *b* and *c* demonstrate at high magnification two sections of nucleoli. The 5-mm gold particles (*small black dots*) indicating gold-antibody-antigen complexes are selectively enriched over the granular component (GC) and are distributed at higher density in the periphery of these nucleoli. The dense fibrillar component (DFC) and the fibrillar centers (FC) are free of gold particles. Note that gold-antibody-antigen complexes are also present in clusters throughout the heterochromatin structures as indicated by arrows in *a* and *c*. Bars 1 μm, ×20,000 (*a*), 0.2 μm, ×70,000 (*b*), 0.2 μm, ×125,000 (*c*).

These electron microscopic findings give support to the observation that in indirect immunofluorescence there is weak nucleoplasmic in addition to nucleolar staining. In the control experiment, antibodies against RNA polymerase I (12) were used that only stained the fibrillar centers but not the granular component of nucleoli.

Effect of actinomycin D and DRB on the distribution of anti-PM-Scl staining. To further study the nature of the PM-Scl antigen, Vero cells were exposed to the nucleolus-segregating drugs actinomycin D and DRB. After drug treatment and fixation, cells were incubated with several anti-PM-Scl sera and stained with a fluorescein anti-human IgG conjugate. Actinomycin D-inactivated nucleoli are compact and show a typical segregation of the fibrillar component from the granular component, usually resulting in the formation of distinct nucleolar hemispheres (15–17). Figure 4 shows the results obtained with prototype serum S125. Actinomycin D at 0.1 μg/ml considerably reduced nucleolar staining by anti-PM-Scl antibodies. Residual staining was predominantly localized in the granular component (Fig. 4*b*), which appeared dark in phase optics (Fig. 4*c*). When DRB was added to Vero cells growing in culture, the normally compact nucleoli unraveled into extended beaded strands, again segregating the nucleolus into granular and fibrillar regions as has been described in previous studies (18–20). Treatment of Vero cells with DRB at 50 μg/ml for 4 to 6 hr substantially removed PM-Scl staining of segregated nucleolar structures (Fig. 4*e* and *f*). However, diffuse nucleoplasmic staining of low intensity (+), which was not reproduced well in the micrograph, was still present. In contrast, a mouse monoclonal IgG2a antibody against a M_r 34,000 nucleolar protein associated with the U3 ribonucleoprotein particle (Reimer et al., manuscript in preparation) still strongly reacted with the fibrillar component of actinomycin D- and DRB-segregated nucleoli (Fig. 4*a* and *d*). These observations show that actinomycin D and DRB significantly affect expression of PM-Scl antigen, leaving another nucleolar protein essentially unaffected.

Nuclease digestion studies. To evaluate the effect of nuclease digestion on PM-Scl antigen, HeLa cells were incubated with DNase and RNase before incubation with anti-PM-Scl serum. Neither of these nucleases substantially altered intensity of nucleolar immunofluorescence staining when compared with controls in which buffers without enzyme were used. In other control experiments, nuclear staining by a monoclonal anti-native DNA antibody (11) was abolished by DNase pretreatment of cells, and nucleolar and cytoplasmic staining produced by human and monoclonal anti-RNA antibodies was abolished by RNase pretreatment. These findings suggested that the antigenicity of PM-Scl is not dependent on DNA or RNA.

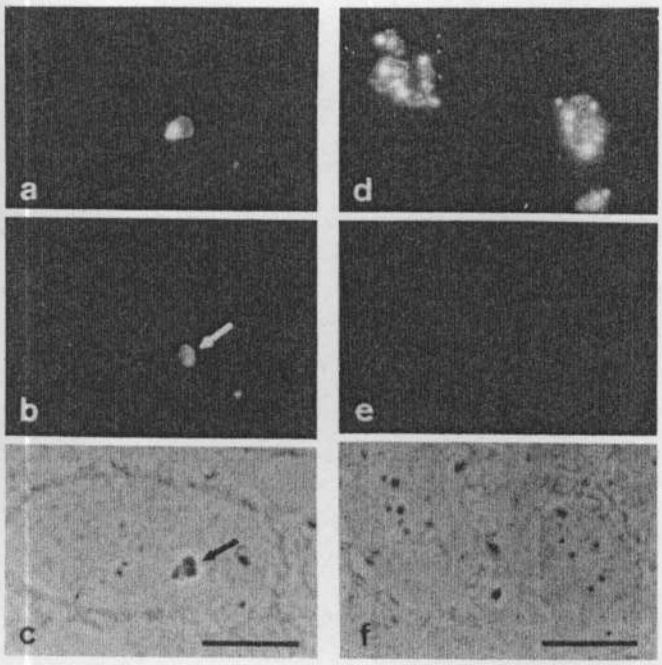

Figure 4. Double immunofluorescence staining. A mouse monoclonal IgG2a antibody against fibrillarin, a basic nucleolar protein, and human anti-PM-Scl antibodies (S125) was reacted simultaneously with actinomycin D- (*a* and *b*) and DRB- (*d* and *e*) treated Vero cells. The IgG2a mouse monoclonal antibody (*a* and *d*) was detected with a specific fluorescein conjugate; the human antibodies, with an anti-IgG rhodamine conjugate (*b* and *e*). Staining intensity of fibrillar components of nucleoli by monoclonal antibody to fibrillarin was not significantly affected by actinomycin D (*a*) or DRB (*d*). In contrast, anti-PM-Scl antibodies that reacted with the granular component (*arrow*) showed considerably reduced staining intensity after actinomycin D treatment (*b*) when compared with untreated nucleoli (for comparison see Fig. 1*c*). DRB treatment almost completely abolished anti-PM-Scl staining of dispersed nucleolar structures (*e*). For reference, cells are also shown in phase-contrast optics (*c* and *f*). Bar 10 μm, ×1450.

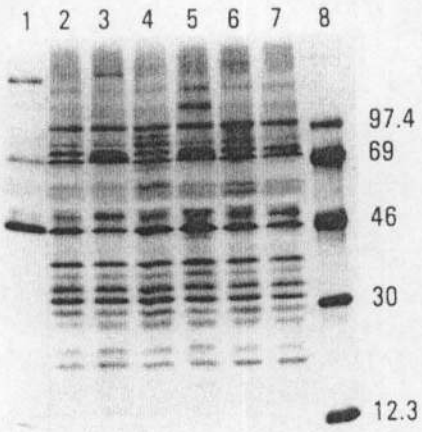

Figure 5. Autoradiogram of immunoprecipitated [^{35}S]methionine-labeled HeLa cell proteins electrophoretically resolved in a 15% SDS-polyacrylamide gel. Lane 1, normal human serum; lane 2 to 7, anti-PM-Scl sera as identified previously in immunodiffusion; lane 8, molecular weight markers. A group of distinct polypeptides were uniformly precipitated by all anti-PM-Scl sera but not by normal control serum.

Molecular characterization of the PM-Scl antigen. To identify the molecular composition of the PM-Scl antigen, we performed protein A-facilitated immunoprecipitation of radiolabeled antigen followed by gel analysis. Immunoprecipitation with six anti-PM-Scl sera, using [^{35}S]methionine-labeled HeLa cell extract, followed by SDS-PAGE analysis are shown in Figure 5, lanes 2 to 7. All sera with PM-Scl specificity by double immunodiffusion uniformly precipitated at least 11 polypeptides (P1-P11) with M_r 110,000, 90,000, 80,000, 39,000, 37,000, 33,000, 30,000, 27,000, 26,000, 22,000, and 20,000. Figure 6, lane 2, shows in detail the polypeptides precipitated by representative anti-PM-Scl serum S125. On closer examination of Figure 5 it can be noticed that there are additional polypeptides present in lanes 3, 4, and 5, but the common polypeptides precipitated by all sera examined comprised the P1-P11 complex as shown in Figure 6.

HeLa cells were labeled with [^{32}P]orthophosphate, and the cell lysate was used in immunoprecipitation to determine whether the PM-Scl antigen might be phosphorylated or associated with tightly bound RNA. The M_r 80,000 (P3) and 20,000 (P11) polypeptides that were precipitated by anti-PM-Scl antibodies were phosphoproteins (Fig. 7, lane 4). RNA was not part of the PM-Scl antigen as shown by absence of RNA in urea-polyacrylamide gels. We also incubated deproteinized RNA from HeLa cells with anti-PM-Scl antibodies but failed to demonstrate any binding with RNA, suggesting that RNA itself is not a target (data not shown). The latter data are in agreement with the in situ nuclease digestion results obtained in tissue culture HeLa cells, where no effect of RNase pretreatment of cell substrates on immunostaining with anti-PM-Scl was observed.

Several attempts were made to identify the antigenic epitopes of the PM-Scl complex by immunoblotting experiments. Extractable whole HeLa cell proteins as well as isolated nucleolar proteins were separated by SDS-PAGE and were transferred to nitrocellulose for immunoblotting. At the present time, no conclusive results have been obtained, although a protein of approximately M_r 80,000 has been reactive with some sera. Various explanations are possible, such as denaturation of the antigens and the requirement of tertiary conformation for antigenicity. Definitive identity of the antigenic component of the PM-Scl complex awaits further study.

DISCUSSION

Antinucleolar antibodies are part of the spectrum of autoantibodies present in scleroderma and were found to

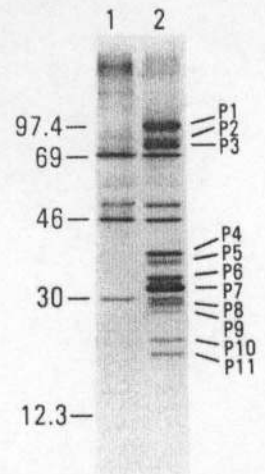

Figure 6. Autoradiogram of [^{35}S]methionine-labeled HeLa cell proteins immunoprecipitated by anti-PM-Scl antibodies (S125) and resolved in a 17.5% SDS-polyacrylamide gel. Lane 1 shows proteins precipitated by normal control serum. Lane 2 demonstrates in detail the radiolabeled proteins from HeLa cells that are selectively brought down by anti-PM-Scl antibodies and are named P1-P11.

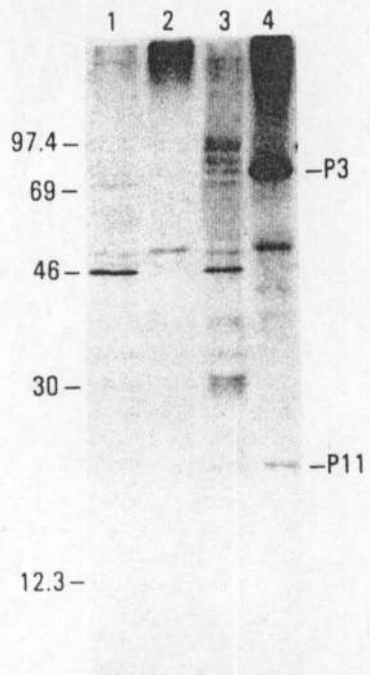

Figure 7. Autoradiogram of immunoprecipitated, [^{35}S]methionine- (lane 1 and 3) and [^{32}P]orthophosphate- (lane 2 and 4) labeled HeLa cell proteins. Immunoprecipitates as obtained with normal human serum are shown in lane 1 and 2 and with anti-PM-Scl serum in lane 3 and 4. The polypeptides of M_r 80,000 (P3) and 20,000 (P11), which are labeled with [^{35}S]methionine, are phosphorylated. The dark smear on top of lane 2 and 4 as well as the signal just above marker of M_r 46,000 were sensitive to RNase treatment and most probably represents coprecipitating RNA in normal human and anti-PM-Scl serum.

be highly associated with this systemic autoimmune disease (21–24). Nucleolar 4-6 S RNA was shown to be one of the targets (24). More recently, a novel nucleolar ribonucleoprotein complexed with 7-2 RNA was detected with antinucleolar scleroderma antibodies (25, 26). U3 ribonucleoprotein was also shown to be part of a complex precipitated by certain antinucleolar antibodies from scleroderma sera (25). These antibodies were shown to recognize a M_r 34,000 basic nucleolar protein rich in N^G, N^G-dimethylarginine, and glycine (27). Because of its predominant localization in the fibrillar component of nucleoli, Ochs et al. (28) named this nucleolar protein fibrillarin. We recently showed that a subgroup of antinucleolar antibodies that produce speckled or punctate nucleolar immunofluorescence staining in tissue culture cells immunoprecipitated the RNA polymerase I complex (29). Naturally occurring autoimmune antibodies against nucleolar proteins and RNA protein complexes thus far have proved to be valuable tools to dissect nucleolus structure and function.

In the present study, we have characterized the antigen reactive with anti-PM-Scl antibodies that were previously shown to be associated with polymyositis, scleroderma, or polymyositis/scleroderma overlap syndromes. The predominant nucleolar presence of the PM-Scl antigen was demonstrated by indirect immunofluorescence performed by using tissue culture cells from a variety of species. The wide distribution of the epitopes recognized by anti-PM-Scl autoantibodies suggests that the reactive protein is a conserved and important cellular component. The nucleolar localization of the PM-Scl antigen as observed in this study is in keeping with a recent report by Targoff and Reichlin (9), who also found this antigen to be nucleolar. However, from our immunolocalization studies it appears that the PM-Scl antigen is also present in the nucleoplasm.

By electron microscopic immunocytochemistry, anti-PM-Scl antibodies predominantly stained the granular component of nucleoli and clustered particles dispersed throughout the heterochromatin. In actinomycin D-segregated nucleoli, anti-PM-Scl staining was substantially reduced but mainly localized in the granular regions. Actinomycin D in the concentrations used in this study suppresses the synthesis of rRNA and the activity of RNA polymerase II but not the synthesis of ribosomal proteins (30–33). In the absence of rRNA synthesis and despite normal protein synthesis, ribosomal proteins are rapidly degraded (32). Another drug, the halogenated adenosine analogue DRB, also had a markedly suppressive effect on the expression of the PM-Scl antigen. This drug almost completely abolished anti-PM-Scl staining in tissue culture cells when added to the medium. DRB is known to effectively suppress the synthesis of mRNA (34–36) but not, or only moderately, transcription of the rRNA genes (37, 38). DRB causes extensive degradation of the RNA precursor particles of the large ribosomal subunit, probably due to the deficiency of essential preribosomal proteins. The production of the small ribosomal subunit appears not to be impaired (18, 19). Therefore, localization of the PM-Scl antigen predominantly in the granular region of nucleoli, the site of ribosome assembly and packaging, and more importantly sensitivity of the PM-Scl antigen to actinomycin D and DRB, drugs that cause degradation of preribosomes, may suggest a relationship of the PM-Scl antigen to preribosomes. The distribution of the PM-Scl antigen during cell cycle is compatible with a possible preribosomal origin or association.

On a molecular level, anti-PM-Scl antibodies immunoprecipitated 11 polypeptides with M_r from 110,000 to 20,000 when incubated with [^{35}S]methionine-labeled HeLa cell proteins from the extractable fraction. The M_r 80,000 and 20,000 polypeptides were phosphorylated. RNA was not precipitated with the PM-Scl antigen, and therefore, no association of the PM-Scl complex with ribosomal precursor RNA or processed rRNA could be established that could have facilitated identification of the putative nature of the PM-Scl antigen. We also attempted to define the antigenic components of the PM-Scl antigen complex recognized by autoimmune antibodies. With the use of extractable HeLa cell proteins as well as isolated nucleoli as antigen source in immunoblotting, the antigenic polypeptides of the PM-Scl complex could not be conclusively revealed.

The PM-Scl antigen, however, is clearly different from the 7-2 ribonucleoprotein particle recognized by certain antinucleolar scleroderma sera (25, 26) since none of our anti-PM-Scl sera coprecipitated 7-2 S RNA. U3 RNA was also not present in the immunoprecipitates of anti-PM-Scl antibodies, suggesting that the U3 ribonucleoprotein is not part of the PM-Scl complex. We also incubated deproteinized RNA with anti-PM-Scl antibodies and showed that RNA was not a target, suggesting that these antibodies are different from those reported to react with 4-6 S nucleolus-specific RNA (24) or any other RNA species.

Polyclonal and monoclonal antibodies have recently been used by several investigators to characterize nucleolar proteins in greater detail. For example, a mouse monoclonal antibody was shown by Hugle et al. (39) to identify the largest and most acidic protein of the small ribosomal subunit (S1) with M_r 43,000 in the rat. Another monoclonal antibody characterized a nuclear M_r 40,000 protein named ribocharin, which appears to be specific to precursor particles of the large ribosomal subunit in *Xenopus laevis* and other amphibia (40). Recently, a M_r 100,000 nucleolar protein was identified as being associated with preribosomes (41). Other nucleolar proteins that have been partially characterized include protein C23 and B23. In several reports, it has been suggested that protein C23 may be a component of the preribosomal ribonucleoprotein particles (42, 43) and protein B23 may be associated with ribosome assembly and packaging (44, 45). It is unclear at the moment whether some of the PM-Scl polypeptides are identical with known nucleolar proteins, but it is conceivable. Further characterization of the PM-Scl complex is needed to answer its exact composition and function in the nucleolus.

Defining the structure and function of the PM-Scl autoantigen may be a first step towards understanding why patients with features of polymyositis and scleroderma elicit an autoimmune response directed against this nucleolar macromolecule.

Acknowledgments. We thank Thomas H. Hoger, Institute of Cell and Tumor Biology, German Cancer Research Institute, Heidelberg, for performing some of the electron microscopy presented in this study. We also thank Dr. Carol A. Penning and Carol L. Peebles, W. M. Keck Foundation Autoimmune Disease Center, Scripps Clinic and Research Foundation, La Jolla, CA, for assisting with some of the experiments in this study.

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