CHARACTERIZATION OF PROLIFERATING CELL NUCLEAR ANTIGEN RECOGNIZED BY AUTOANTIBODIES IN LUPUS SERA

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In a small percentage of patients with systemic lupus erythematosus (SLE), an autoantibody that is reactive with a nuclear antigen in proliferating cells (PCNA) has been demonstrated (1). Using this autoantibody as the reagent in immunofluorescence, PCNA was not detected in differentiated, nondividing cells such as renal tubular or glomerular cells nor in hepatic parenchymal cells. However, continuous tissue culture cell lines of different species such as WiL-2, Hep-2, baby hamster kidney cells, and Ehrlich ascites tumor cells were positive for PCNA. Normal peripheral blood lymphocytes did not contain detectable amounts of PCNA, but it was present in some lymphocytes in lymph node follicles, spleen, and thymus. After mitogenic stimulation, peripheral blood lymphocytes became positive for PCNA, demonstrable as nuclear staining in the blast-transformed cells.

The reactive nuclear antigen was extractable from tissue culture cells or rabbit thymus with physiological saline. Solubilized PCNA formed a precipitin line with autoantibody that was immunologically distinct from previously identified antigen-autoantibody systems such as Sm antigen, nuclear RNP, SS-A/Ro, and SS-B/La (2).

The incidence of anti-PCNA antibody is <5% in SLE patients and it does not appear to be associated with any distinctive clinical characteristics in such patients (1). However, because of the apparent association of PCNA with proliferation and blast transformation, it has been used as a probe to detect blast crisis in patients with leukemia. In this report we describe methods for purification of PCNA and some of the biochemical characteristics of the antigen.

Methods

Preparation of Specific Anti-PCNA Serum. Three anti-PCNA sera (MN, EB, and PT) obtained from patients with SLE were used. In addition to anti-PCNA, these sera

This is publication 3304BCR from the Department of Basic and Clinical Research. It was supported by grants AM30263 and AI10036 from the National Institutes of Health. Present addresses: Y. T., Juntendo University, School of Medicine, Tokyo, Japan; D. F., Stanford University, Palo Alto, CA.

Abbreviations used in this paper: CIE, counterimmunoelectrophoresis; NCP, nitrocellulose paper; PBS, phosphate-buffered saline; EBa, absorbed EB sera; PCNA, proliferating cell nuclear antigen; PTa, absorbed PT serum; RTA, rabbit thymus extract; SDS, sodium dodecyl sulfate; SLE, systemic lupus erythematosus.

Takasaki, Y., W. A. Robinson, and E. M. Tan. Proliferating cell nuclear antigen (PCNA) in blast crisis cells of patients with chronic myeloid leukemia. Manuscript in preparation.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/84/04/0981/12 $1.00 981

Volume 159 April 1984 981-992
contained autoantibodies to other nuclear antigens including double-strand DNA, histone, Sm, and SS-A/Ro (see Table I). Anti-PCNA sera were rendered monospecific by the following procedure.

It was previously observed (1) that differentiated tissues such as rabbit kidney contained negligible amounts of PCNA but contained good concentrations of other antigens such as Sm and U1-RNP (1). Rabbit kidney extract was therefore used as a source of antigen(s) to absorb other antibodies from EB and PT sera as previously described (3). Rabbit kidney was homogenized with a Potter homogenizer in 0.15 M NaCl (1 g rabbit kidney/4 ml 0.15 M NaCl), and the suspension was sonicated for six 15-s cycles at maximum output with a model W185 sonicator (Heat System-Ultrasonics, Inc., Plainview, NY). The sonicate was stirred for 4 h at 4°C and centrifuged at 3,500 g for 20 min. The supernatant, containing ~60 mg of protein/ml, was used as a source for making solid-phase immunoadsorbant as described by Avrameas and Ternynck (4). 4 ml of rabbit kidney extract was mixed with 4 ml of bovine serum albumin (60 mg/ml), the latter being used as a nonspecific protein carrier. To this mixture, 0.8 ml of 2 M acetate buffer, pH 5, was added, followed by 2 ml of 2.5% glutaraldehyde added dropwise. The reaction mixture was allowed to stand for 4 h at room temperature without stirring. After gelation, it was homogenized with a Potter homogenizer and dispersed in 50 ml of 0.1 M phosphate buffer, pH 7.4. After centrifugation, the immunoadsorbant gel was washed repeatedly with phosphate buffer until the OD 280 nm of the supernatant was <0.05. The gel was then rehomogenized, dispersed in 50 ml of 0.2 M HCl-glycine, pH 2.8, and stirred for 15 min at room temperature. The gel was subsequently neutralized with 1 M K2HPO4, centrifuged and dispersed in 10 ml of 0.1 M lysine, and incubated overnight at 4°C. For use as immunoadsorbant, the gel was washed in phosphate-buffered saline (PBS) and resuspended in PBS containing 0.01% sodium azide. 1 ml of anti-PCNA serum (EB or PT) was diluted 1:10 with PBS, added to 30 ml of packed gel, and incubated for 1 h at room temperature and for 24 h at 4°C. After this incubation, the mixture was centrifuged at 3,500 g for 30 min and the supernatant was recovered and concentrated to the original volume of anti-PCNA serum with a Minicon B125 concentrator (Amicon Corp., Bedford, MA).

Preparation of Tissue Antigen. Rabbit thymus extract (RTE) was used as the source material containing PCNA. The extract was prepared as previously reported (5). For each 90 mg of rabbit thymus acetone powder (Pel-Freeze Biologicals, Rogers, AR), 1 ml of PBS (0.15 M NaCl, 0.01 M phosphate buffer, pH 7.4) was added. Saline-soluble antigen was extracted by stirring this mixture at 4°C for 24 h. The mixture was centrifuged at 3,000 g for 20 min and the supernatant was used as the source of antigen, RTE. Protein concentration of RTE was ~18–21 mg/ml by the Lowry procedure (6).

Double Immunodiffusion and Counterimmunoelectrophoresis. The double diffusion method was used to demonstrate and identify the precipitin reaction between crude or purified antigen and specific antibodies as previously described (7). Counterimmunoelectrophoresis (CIE) was also used to detect the antigen during its purification and to determine the extent of antigen purification by ascertaining the minimum amount of protein required to yield a precipitin line with the same antiserum. The procedure has been previously described (5).

Rocket Immunoelectrophoresis. Rocket immunoelectrophoresis (8) was used to determine the amount of PCNA in preparations of crude or purified antigen. 8 ml of 1% agarose in 0.025 M barbital buffer (50–55°C) containing anti-PCNA serum were pipetted on to glass slides (5 × 7.5 cm). After the agarose congealed, 3-mm diam wells were punched out 3 mm apart and used as the cathodal set. 20 µl of sample was added into each well. Electrophoresis was performed in 0.05 M barbital buffer, pH 8.4, with a current of 1.5 mA/cm for 4 h at 4°C. After electrophoresis, the slide was incubated at room temperature for 24 h, washed in PBS for 2 d with at least two changes, dried, and stained with 0.1% amido black 10B in 10% ethanol/1% glacial acetic acid (Sigma Chemical Co., St. Louis, MO). The heights of the rocket peaks were measured to determine the relative amount of PCNA in each sample.

Antigen Purification. Saturated ammonium sulfate solution was prepared by heating...
1,000 g ammonium sulfate in ~1 liter water at 50°C until most of the salt was dissolved. It was allowed to stand for 3 d at 4°C after which the pH was adjusted to 7.0 by the addition of NH₄OH. Before fractionation with ammonium sulfate, RTE was centrifuged at 105,000 g for 1 h. The supernatant was used as an antigen source. To determine the percentage of ammonium sulfate needed to precipitate the maximum amount of PCNA from the crude extract, saturated ammonium sulfate solution was added dropwise to 2-ml aliquots of centrifuged RTE (protein concentration, 16 mg/ml). The mixtures were stirred at 4°C for 4 h, then centrifuged at 8,000 g for 20 min and the supernatants discarded. Precipitates were dissolved in minimum amounts of PBS and dialyzed against >100 vol of the same buffer for 24 h with at least one change. After dialysis, the volume was adjusted to the original volume of starting RTE. The relative amount of PCNA was compared by rocket immunoelectrophoresis and by titration in double immunodiffusion.

DEAE-cellulose (DE 52 preswollen; Whatman Laboratory Products, Inc., Clifton, NJ) was equilibrated with 0.01 M phosphate buffer, pH 8.0, and packed in a column (1.5 × 20 cm). A stepwise salt gradient, in which 0.1–1 M NaCl were added to starting buffer (0.01 M phosphate buffer, pH 8.0), was used for fractionation. PCNA purified by ammonium sulfate fraction was applied to the column and eluted with a stepwise NaCl gradient in 0.1 M increments.

Sephadex G200 (Pharmacia Fine Chemicals, Piscataway, NJ) columns of 1.5 × 90 cm were used for further purification and molecular weight determinations. PBS was used as the running buffer. 1.5 ml of sample was applied and eluted at a flow rate of 3 ml/h. Blue dextran (2,000,000 mol wt), human IgG (150,000), bovine serum albumin (66,200), ovalbumin (45,000), and soybean trypsin inhibitor (21,500) were used as molecular weight markers.

Enzyme Digestion Study and Sucrose Density Gradient Ultracentrifugation. Partially purified PCNA from G200 columns was treated with deoxyribonuclease (DNase I; Millipore Corp., Freehold, NJ), ribonuclease (RNase A; Millipore Corp.), and trypsin (Miles Laboratories, Elkhart, IN). The ratio of enzyme to substrate was 1:10 by weight as previously described (9). The enzyme was made up in PBS and incubated with a fivefold greater volume of substrate for 1 h at 37°C. DNase digestion was performed in PBS with 6 mM MgCl₂. Partially purified PCNA was sensitive to trypsin and resistant to DNase and RNase as shown below. The molecular weight of nondigested and enzyme-digested PCNA was analyzed by sucrose density gradient ultracentrifugation. Continuous gradients were made with 5 and 20% ultrapure sucrose (Schwarz Mann, Orangeburg, NY) in PBS. 50 μl of partially purified PCNA and enzyme-digested PCNA were mixed with 150 μl of PBS and layered on top of the gradient, spun at 125,000 g for 24 h at 4°C, and collected dropwise from below. PCNA activity in the fractions was determined by CIE and rocket immunoelectrophoresis.

Isoelectric Focusing. PCNA-active fractions from G200 Sephadex columns were pooled, concentrated, and assessed by isoelectric focusing in purified agarose (Isofet; Marine Colloid Bioproducts, Rockland, ME). A 1% agarose solution was incubated in a 65°C water bath for 20 min and amphotoline pH 4–6.5 (Pharmacia, Uppsala, Sweden) was added to final concentration of 1%. Isoelectric focusing was carried out at 600 V for 8 h at 4°C. The gel was stained with 0.05% Coomassie blue in 50% methanol/10% acetic acid and destained.

Preparation of IgG and Immunoprecipitation. IgG of anti-PCNA serum (absorbed PT or EB) was purified by ammonium sulfate precipitation followed by DEAE-cellulose (DE 52) column chromatography essentially as described by Heide and Schwick (10) and Fahey and Terry (11). Immunoprecipitation was performed as follows. 500 μl of IgG purified from absorbed EB (16.4 mg) or absorbed PT (9.2 mg/ml) was added to 50 μl of partially purified PCNA obtained from the G200 fraction (~120 μg protein/ml), then incubated for 1 h at room temperature and 72 h at 4°C. The mixture was centrifuged at 2,500 g for 15 min. The precipitate was washed in PBS three times and in 0.001 M phosphate buffer once. After washing, the precipitates were prepared as described below for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis in SDS. Polyacrylamide slab gel electrophoresis in SDS
TABLE I

Characteristics of Sera Containing Antibody to PCNA

| Serum | Antibodies to: |
|-------|---------------|
|       | PCNA         | DS-DNA* | Histone | Sm | SS-A/Ro |
| MN†   | +           | -       | +       | -  | -       |
| PT    | +           | -       | -       | +  | +       |
| EB    | +           | -       | -       | +  | -       |

* Double-strand DNA.
† In MN serum, antibodies to DNA and histone were present in low concentration whereas antibody to PCNA was present in high concentration. At higher dilutions of the serum, MN was operationally "monospecific" for PCNA.

was performed according to the method of Weber and Osborn (12) and Laemmli (13). The gels were formed in 1.5 x 160 x 140 mm slabs that consisted of 7.5 or 10% acrylamide (wt/vol) separating gels in 0.1% SDS and Tris-HCl, pH 8.8, with 1-cm stacking gels containing 5% (wt/vol) acrylamide in 0.1% SDS and Tris-HCl, pH 6.8. Samples were dissolved in sample buffer (3% SDS, 5% 2-mercaptoethanol, 55 mM Tris-HCl, pH 6.8, 10% glycerol, bromphenol blue) or in sample buffer without 5% 2-mercaptoethanol by boiling for 3 min. Samples applied to the gel were stacked at 1 mA/cm, run at 2-3 mA/cm for 4-5 h, and fixed and stained with 0.04% Coomassie brilliant blue in 25% isopropyl alcohol/10% acetic acid overnight.

**Electrophoretic Blotting.** Partially purified PCNA was first subjected to electrophoresis in 10% SDS-polyacrylamide slab gel and transferred to nitrocellulose paper (NCP) according to the method of Towbin et al. (14). All manipulations were performed at room temperature after NCP strips (0.4 μm pore size; Millipore Corp.) were soaked in Western blot electrode buffer (192 mM glycine, 25 mM Tris base, 20% ethanol, pH 8.3) and placed on the gel strips. The gel and NCP strips were placed between sponge pads that were saturated with electrode buffer. The proteins were electrophoretically transferred at 12 V for 3 h in electrode buffer. After electrophoresis, NCP strips were separated into two groups. One was stained by amido black (0.1% in 45% methanol, 10% acetic acid) to determine the profile of proteins transferred to the NCP strips. The other was used for immunological detection of PCNA. The unstained electrophoretic blots were rinsed once in Western blot saline (0.9% NaCl, 10 mM Tris-HCl, pH 7.4, 0.025% NP-40) and incubated for 15 h with 1% (wt/vol) bovine serum albumin (radioimmunoassay grade; Sigma Chemical Co.) in Western blot saline to saturate additional sites of nonspecific protein binding. They were rinsed in Western blot saline once and incubated with anti-PCNA serum (PTa, 1:100 dilution) as well as anti-Sm anti-SS-A serum (RC, 1:40) and normal human serum (1:40) for 4 h. To remove unbound antibody, NCP strips were washed for 1 h with at least three changes of Western blot saline. Affinity-isolated goat anti-human IgG antibodies (Tago, Inc., Burlingame, CA) labeled with 125I was used as the detecting reagent. Strips were rinsed in Western blot saline, dried for 6 h, and exposed to Kodak X-Omat R film at -20°C for 4 d with a Kodak hi-plus intensifying screen.

**Results**

**Preparation of Specific Anti-PCNA Serum.** Our analysis demonstrated that three sera, MN, PT, and EB contained other antibodies in addition to anti-PCNA (Table I) and absorption studies were performed with the insolubilized immunoadsorbant of rabbit kidney extract to render them "monospecific." In double immunodiffusion analysis (Fig. 1A), EB and PT reacted with RTE to produce two precipitin lines. After absorption with kidney extract, absorbed sera PT (PTa) and EB (EBa) showed only one precipitin line that was identical to serum
FIGURE 1. Analysis of antibodies in sera used as reagents. (A). In immunodiffusion, MN serum reacted with RTE to give a single precipitin line that had previously been shown to be anti-PCNA. However, sera EB and PT (unabsorbed) showed two precipitin lines, the outer lines fusing in immunological identity with precipitin of reference serum TT, known to contain anti-Sm antibody. After absorption of EB and PT sera with kidney extract (EBa and PTa), the Sm antibody was removed and only anti-PCNA remained in the absorbed sera. (B) In counterimmuno-electrophoresis, the immunological identity of precipitins between MN serum and PTa and between PTa and EBa was also confirmed. Nonidentity between EBa and TT was demonstrated by the crossing-over of the precipitins.

MN precipitin. The characteristics of the MN precipitin line which established that this was anti-PCNA had been published previously (1). The second line demonstrated by PT and EB was anti-Sm since it completely fused in immunological identity with the reference anti-Sm serum TT. The absorbed sera were also analyzed by CIE (Fig. 1B). Anti-PCNA sera MN, PTa, and EBa and anti-Sm sera TT were in the anodal wells; RTE (containing PCNA) was in the cathodal wells. This technique, which is more sensitive than double immunodiffusion, also demonstrated the identity of PTa and EBa with MN and showed the absence of other precipitating antibodies. In a fashion characteristic of sera containing anti-PCNA but devoid of other types of antinuclear antibodies, EBa or PTa did not react with nuclei of isolated peripheral blood lymphocytes but reacted strongly with the nuclei of mitogen-stimulated cells (Fig. 2). The monospecificity of EBa and PTa was further confirmed by immunofluorescence studies on other substrates such as mouse kidney. Before absorption, both sera showed weak staining of renal tubular cells, presumably due to anti-Sm and perhaps other antinuclear antibodies. After absorption, these sera were negative on renal tubular cells. The studies reported subsequently were performed primarily with PTa and EBa. Due to limited quantities of MN serum that was monospecific at serum dilutions of 1:160 and higher (see Table I), it was used only at certain points to confirm the findings.

Purification of PCNA. With RTE as the starting material, the first step of purification consisted of determining the optimum concentration of ammonium sulfate for its precipitation. By rocket immunoelectrophoresis, no PCNA was precipitated below 40% ammonium sulfate (Fig. 3). Increasing amounts were obtained until 65% saturation; for batchwise first-step purification, the precipitate between 40 and 65% saturation was used. This latter material was identical immunologically with starting RTE, as demonstrated in Fig. 3B. The second step in purification used DEAE chromatography with stepwise increasing concentrations of NaCl for elution. PCNA activity, determined by CIE and rocket immunoelectrophoresis was eluted fairly discretely with 0.25 M NaCl, 0.01 M
FIGURE 2. (A) When EBa and PTa sera (absorbed sera to render them "monospecific" for PCNA) were used to stain peripheral blood lymphocytes in immunofluorescence, no nucleolar staining was observed. The apparent cytoplasmic fluorescence was due to autofluorescence and longer film exposure. (B) PHA or concanavalin A stimulation induced expression of PCNA in lymphocytes so that there was strong nuclear fluorescence after reaction with PTa and EBa sera.

FIGURE 3. Ammonium sulfate precipitation was the first step used in purification of PCNA. (A) Rocket immunoelectrophoresis was used to measure semiquantitatively the amount of PCNA in precipitates. Practically all PCNA in RTE was precipitated at between 40 and 65% ammonium sulfate saturation. (B) The material precipitated (40–65 P) showed immunological identity with crude RTE and RTE clarified by centrifugation (RTE cent.). The precipitate at 40% ammonium sulfate and the supernate at 65% did not contain detectable amounts of PCNA.

phosphate, pH 8.0 (Fig. 4). This material was then dialyzed against phosphate-buffered normal saline (0.15 M NaCl, 0.01 M phosphate, pH 7.4) and applied to a G200 column (Fig. 5). PCNA activity was detected over a wide range of elution fractions but the peak of activity was ~100,000 mol wt in reference to
FIGURE 4. The second step in purification used DEAE column chromatography. Starting material was 40–65% ammonium sulfate precipitate. PCNA activity was eluted with 0.25 M NaCl.

FIGURE 5. The 0.25 M NaCl from DEAE was concentrated and analyzed on Sephadex G200 columns. PCNA activity was detected over a wide range of elution fractions but the peak activity was in the range of 100,000 mol wt.

the markers used. Using rocket immunoelectrophoresis as a semiquantitative measure of activity, the G200 material was concentrated 120-fold over starting RTE (Table II). The G200-derived PCNA retained complete immunological identity with PCNA in starting RTE as determined by double immunodiffusion.

**Properties of Partially Purified PCNA.** G200-derived PCNA activity was destroyed by trypsin digestion but was resistant to DNase and RNase A, strongly
TABLE II
Comparison of Quantitative PCNA Activities Among Crude and Partially Purified Fractions

| Protein concentration | Rocket immunoelectrophoresis | Purification (fold) |
|-----------------------|-----------------------------|---------------------|
| mg/ml                 |Rocket immu |                  |
| Starting RTE          | 16.8  | 1    | 1 |
| Ultracentrifugation   | 15.5  | 0.93 | 1.008  |
| Ammonium sulfate fractionation | 11.6  | 2.21 | 3.2 |
| DE-52 column          | 1.32  | 1.5  | 19 |
| G200 column           | 0.122 | 0.87 | 120 |

* Ratio of height of rocket observed after various procedures compared with crude extract (RTE).

suggesting that the antigen was a nuclear protein. Its activity was lost after incubation at 56°C for 30 min. In sucrose density gradient ultracentrifugation, the peak of activity was recovered in the 94,000–100,000 Mw range (Fig. 6), a finding consistent with G200 filtration data. In isoelectric focusing, the peak activity was at pH 4.8.

Polypeptide Composition of PCNA. The G200-derived PCNA was heterogeneous in composition, showing at least seven polypeptide bands in polyacrylamide gel electrophoresis (Fig. 7, lane 5). This material was used as the antigen source in spontaneous immunoprecipitation with PTa and EBa sera (see Methods) and the immunoprecipitates were analyzed by gel electrophoresis in the presence of 2-mercaptoethanol. With either PTa or EBa (lane 4), a single protein of ~33,000 Mw was immunoprecipitated. Lanes 1 and 2 show that only heavy and light
When G200-derived PCNA was analyzed in reduced polyacrylamide gels, at least seven protein bands were detected (lane 5). IgG alone from PTa and EBa showed the expected heavy and light chains (lanes 1 and 2). Immunoprecipitates of IgG and G200 material showed that in addition to heavy and light chains, a protein of 33,000 mol wt was present (lanes 3 and 4). Molecular weight markers are shown in lane 6.

To confirm the above findings immunoblotting was performed with the G200-purified PCNA. In reduced (with 2-mercaptoethanol) or nonreduced gels several proteins were transferred to nitrocellulose paper (lanes 2 and 3, respectively, Fig. 8). When parallel strips were reacted with PTa serum, only the 33,000 mol wt band reacted. This is illustrated in Fig. 8, lane 4 for the reduced gel transfer and in lane 5 for the nonreduced gel transfer. Lanes 6 and 7 show that parallel strips reacted with a serum containing antibodies to Sm and SS-A/Ro, demonstrating the lack of reactivity of the 33,000 mol wt protein with other antinuclear antibodies.

**Discussion**

Our approach to the characterization of PCNA has been to use standard biochemical techniques to achieve partial purification and to use immunoprecipitation and gel electrophoresis techniques to identify the antigen. The combination of ammonium sulfate precipitation, DEAE ion exchange chromatography and G200 gel filtration resulted in a preparation of antigen that was purified 120-fold over the original starting material from RTE. In both immunoprecipitation and electrotransfer immunoblotting assays, the reactive antigen was a 33,000 mol wt polypeptide. This was consistent with the data showing that the reactivity of the antigen was destroyed by proteolytic treatment but was intact after deoxyribonuclease and ribonuclease A treatment, suggesting that the antigenic moiety resided in a protein component.

Other studies, including G200 gel filtration and sucrose density gradient
ultracentrifugation showed that the approximate molecular weight of the reactive antigen was 100,000. Although this appears to be a discrepancy with the immunoprecipitation and immunoblotting data that show the antigen to be 33,000 mol wt recent information related to the purification of other nuclear autoantigens such as Sm and U1-RNP (15, 16) may help to clarify this issue. These studies showed that nuclear antigens exist as particulate complexes in association with other proteins and with nucleic acids such as small nuclear RNA. It is likely that PCNA is also complexed with other proteins and with one or more species of RNA and that these complexes, under nondissociating conditions such as gel filtration and sucrose ultracentrifugation, manifest molecular weights that are the result of protein-protein or protein-nucleic acid complexes.

Since our initial report (1) of a nuclear antigen present in high concentration in proliferating or blast-transformed cells, there have been other reports that have some relationship to this subject. Bravo et al. (17) and Bravo and Celis (18) have analyzed, by two-dimensional gel electrophoresis, $^{35}$S-methionine- and $^{32}$P-orthophosphate-labeled polypeptides synthesized by spontaneously cycling or virus-transformed cells. Several nuclear polypeptides were identified whose concentration was increased as cells progressed into the S phase of the cell cycle. One of these was a 36,000 mol wt polypeptide called cyclin. Riddle et al. (19) also studied synthesis of cellular proteins in mouse 3T3 cells at different times during cell growth and described certain proteins preferentially stimulated during cell growth, one of which had a 33,000 mol wt. It is possible that one or both of these proteins could be related to or identical with PCNA, and efforts are underway to determine this. Barque et al. (20) have characterized a nuclear antigen related to the S phase of the cell cycle that is a protein of 55,000 mol wt.
and therefore different from PCNA. Gerdes et al. (21) produced a mouse monoclonal antibody, Ki-68, to a nuclear antigen also related to the proliferative phase of the cell cycle. Ki-68 reacted with nuclei in tissues and cultured cell lines in a manner quite similar to that for anti-PCNA. However, the chemical characterization of Ki-68 has not been described.

The value of antibodies to antigens associated with blast transformation, either induced by experimental maneuvers or obtained as spontaneously occurring autoantibodies in human diseases, lies their potential value as specific reagents. These reagents could be used as probes to study processes related to blast transformation or uncontrolled proliferation and as diagnostic markers to detect changes in malignant states. In a recent study using anti-PCNA as the reagent, it was possible to show that certain peripheral blood cells during the blast crisis of patients with chronic myeloid leukemia contained nuclear PCNA. These cells were blastoid cells but, in addition, cells with morphology of myelocytes and metamyelocytes contained PCNA. There was, however, a differential concentration of PCNA, with the myelocytic cells containing lower amounts than blast cells. This suggests that reagents such as anti-PCNA might be used to determine quantitatively the degree of transformation of a cell. The potential usefulness of anti-PCNA for these types of studies needs to be explored further.

Summary

A nuclear antigen associated with cell proliferation (proliferating cell nuclear antigen [PCNA]) and blast transformation is recognized by autoantibodies in the sera of some patients with systemic lupus erythematosus. Using this autoantibody as a reagent, PCNA was purified 120-fold by ammonium sulfate fractionation, DEAE chromatography, and Sephadex G200 gel filtration. The antigenicity of PCNA was sensitive to trypsin but resistant to ribonuclease and deoxyribonuclease, suggesting that the antigenic determinant resided in protein and not nucleic acids. PCNA was inactivated at 56°C for 30 min. Isoelectrophoretic focusing showed that the pI was 4.8. Analysis of immunoprecipitates on polyacrylamide gels showed the presence of IgG heavy and light chains and a single polypeptide band of 33,000 mol wt. This polypeptide band was the reactive antigen in immunoblotting (Western transfer) assays.

Received for publication 5 December 1983.

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