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Identification of an Autoantibody against the Proliferating Cell Nuclear Antigen from a Patient with Systemic Lupus Erythematosus

Tsai-Ching Hsu[1], Joung-Liang Lan[2], Shao-Hsuan Kao[3, 4], Sin-Lun Li[3], Yin-Chang Liu[5#], Chih-Yang Huang[6, 7, 8#], and Bor-Show Tzang[3, 4#]

[1]*Institute of Immunology, Chung Shan Medical University, Taichung*
[2]*Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine, Taichung Veterans General Hospital and National Yang-Ming University, Taipei*
[3]*Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung*
[4]*Clinical Laboratory, Chung Shan Medical University, Taichung*
[5]*Institute of Molecular Medicine, National Tsing-Hua University, Hsin-Chu*
[6]*Graduate Institute of Chinese Medical Science*
[7]*Institute of Basic Medical Science, China Medical University, Taichung*
and
[8]*Department of Health and Nutrition Biotechnology, Asia University, Taichung*
Taiwan, Republic of China

Abstract

Antibodies against the proliferating cell nuclear antigen (PCNA) was first discovered in the sera of systemic lupus erythematosus (SLE) patients. However, the reactivity and specificity of anti-PCNA autoantibodies are still unclear. To investigate the property of anti-PCNA autoantibodies, we conducted an ELISA screening of the anti-PCNA autoantibodies in sera of SLE patients. Eighteen out of 191 SLE sera were found to be positive for anti-PCNA antibodies giving a frequency of nearly 10%. Among the positive sera, a sample with the highest titer of anti-PCNA autoantibody preferentially recognizes the wild-type PCNA as compared to the Y114A mutation which contains a single amino acid substitution at 114 and fails to form the toroidal structure. Moreover, the autoantibody purified from this serum identifies only the free PCNA in crude mammalian cell extracts but not other associated cellular components. This finding raises a possibility that immunostaining with the human anti-PCNA autoantibodies in previous studies might have only partially PCNAs in tissues.

Key Words: autoantibodies, systemic lupus erythematosus (SLE), proliferating cell nuclear antigen (PCNA), toroidal structure

Introduction

Proliferating cell nuclear antigen (PCNA), also known as an auxiliary factor of DNA polymerase δ, was first identified by autoantibodies in sera of systemic lupus erythematosus (SLE) patients. PCNA is highly conserved in various eucaryotic organisms ranging from yeast to human. PCNA is required for both DNA synthesis and DNA excision repair in eukaryotes (11, 13, 16, 17, 19, 22, 25). The protein, an analogue of δ subunit of DNA polymerase III of *E. coli*, forms a trimeric ring around the DNA strands

Corresponding author: Bor-Show Tzang, Ph.D., Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung 40201, Taiwan, Republic of China. Tel: +886-4-23248168, +886-4-24730022 ext. 11680, E-mail: bstzang@csmu.edu.tw
[#]Yin-Chang Liu, Chih-Yang Huang, and Bor-Show Tzang contributed equally to this work.
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to assist DNA polymerase δ in DNA replication (15). In addition to its interactions with components of the DNA synthesis machinery, PCNA has also been found to interact with cell-cycle regulators such as cyclin D, $p21^{(Waf1/Cip1)}$ and Growth Arrest DNA Damage (Gadd)-45 (7, 8, 14, 18, 26, 32).

The frequency of SLE patients whose sera contain anti-PCNA antibodies is about 3% determined by counter-immunoelectrophoresis and immunoprecipitation (2, 19). The epitopes of anti-PCNA autoantibodies have also been studied. A previous study using limited proteolysis has indicated that both C- and N-terminal halves of PCNA are essential for the recognition of the autoantibodies (21). Investigation using C-terminal truncated protein synthesized *in vitro* and mutants forms of PCNA expressed as fusion protein suggested complex heterogeneity of human autoantibodies and epitope dependence on conformation (10). The sulfhydryl groups in the PCNA protein are involved in the antigenicity as PCNA treated by the thiol-modifying agent thimerosal was unable to be recognized by the autoantibodies (31). Recently, studies by Brand *et al.* and Szüts *et al.* (3, 27) show that autoantibodies to PCNA belong to two classes: one consisting 80% of the positive sera recognition region of which depends on nearly the full-length of the PCNA protein, and the recognition region of the other recognition region seems to be more confined.

In this study, while studying the frequency of Chinese SLE patients who produce the anti-PCNA autoantibodies, we have identified a serum sample with a high titer of anti-PCNA autoantibody. The anti-PCNA autoantibody of this serum exhibits preferential recognition of wild-type PCNA as compared to the mutant Y114A which differs from its native counterpart by a single amino acid residue. Furthermore, the purified autoantibody recognizes PCNA which is not associated with other components in the cell extract.

Materials and Methods

Sera

Serum samples were obtained in 1995 from SLE patients of the affiliated hospital of Chung Shan Medical and Dental College or the Veteran General Hospital in Taichung. All patients willing to participate were accepted without exclusion and diagnosis was made by a single board-certified physician. Control sera were obtained from healthy individuals who were mainly students of Tsing-Hua University or Chung-San Medical and Dental College.

Construction of PCNA Expression Vector and Cell Transformation

Wild-type PCNA expression vector was constructed using a rat PCNA cDNA-containing plasmid, PCNA/pGEM-1 (16), as the DNA template in the polymerase chain reaction (PCR). The forward and reverse primers were 5'GCCGGATCC**ATG**TTTGAGGCA3', 5'CCCGTCGACCAACGCC**TAA**GA3', respectively, in which the initial and stop codons are in bold face and the restriction enzyme sites (BamHI and SalI sites) for facilitating cloning are underlined. The PCR product was purified and inserted into a prokaryotic expression plasmid, pET-30a (+) (Novogene, Madison, WI, USA). Similar schemes were used to construct the mutant PCNA expression vector with a plasmid containing the mutant human cDNA, Y114A. (12). The BL21 (pLys) strain of *E. coli* was used as the bacterial host for expressing the recombinant proteins.

Western Blotting

Western blotting was performed as described (22, 24). Proteins were separated on 12.5% SDS-PAGE and subsequently transferred to a nitrocellulose membrane. PCNA protein in the membrane was recognized by the mouse monoclonal antibody to PCNA (Ab-1,Oncogene Science, Uniondale, NY, USA). Goat anti-mouse IgG conjugated with horseradish peroxidase (Santa Crutz Biotechnology, Santa Crutz, CA, USA) was then used to detect the Ag-Ab complexes with an ECL detection kit (Amershanm, Freiburg, Germany).

ELISA

PCNA is a highly conserved protein as can be seen by the amino acid sequence homology between mammalian PCNAs as well as its structure and functions (20). To obtain sufficient quantities of PCNA protein for ELISA screening, a PCNA expression vector was constructed and introduced into *E. coli* as described above. Expression of the recombinant protein was induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG) and purified using a His-tag affinity column. In the procedure, 0.5 μg of recombinant PCNA was immobilized on the surface of each sample well of 96-well plates. To each sample well, 2 μl of human serum was added to the reaction mixture in 1:100 dilution and incubated at 37°C for 1 h. After the incubation period, supernatant of each well was removed and each sample well was washed with excess water. An aliquot of 200 μl of 1,000-fold diluted anti-human IgG (KPL, Gaithersburg, MD, USA) was added as the secondary antibody, and the mixture was incubated at 37°C for 1 h followed by excess washing with water prior to the color reaction. The secondary antibody was conjugated with peroxi-

dase. The color reaction was done with the substrate ABTs [2, 2'-Azinobis(3-ethylbenzthiazoline-sulfonic Acid)] in the presence of H_2O_2 (0.002%) at room temperature for 15 min before measurements of optical density at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Preparation of Monospecific Anti-PCNA Autoantibody

A SLE serum sample which exhibited high titers of anti-PCNA in ELISA was used to prepare the monospecific anti-PCNA autoantibody. Purification was performed using a column of Sepharose-4B immobilized with rat PCNA protein. The bound IgG on the affinity column was eluted by 0.1 N HCl. The elute was collected in 1-ml fractions containing 0.1 ml of 1 M Tris-Cl, pH 8.0.

Indirect Immunofluorescence

Fixed HEp-2 cells were provided by MRL Co Aliquots of diluted sera (1:320 dilution) or purified anti-PCNA autoantibodies (1:80 dilution) were added to the fixed cells. The cells were incubated in a moist chamber at 25°C for 30 min, then washed in 1 × PBS for 5 min. After rinsing the cells with distilled water, the fluorescein isothiocyanate-conjugated antibody was added to the cells. The slides were incubated as before while shielded from excess light. The cells were counterstained with Evans Blue. After rinsing with 1 × PBS, the cells were mounted and analyzed using a Nikon fluorescence microscope.

Immunodiffusion

A double diffusion method was performed as previously described (1). In the experiment shown in Fig. 4B, 20 µg of PCNA (wild type or Y114A) was used in the center wells and 20 µl of sera in the surrounding wells. In the experiment shown in Fig. 5, all the antigens except PCNA were obtained from a commercial source (MBL Company, Nagoya, Japan) and optimal concentrations were used according to the suggestion of the manufacturer. The reaction was incubated at room temperature for 72 h.

Metabolic Labeling and Immnuoprecipitation

CHO-K1 cells at log phase were labeled with ^{35}S-labelled methionine (50 µCi per 10-cm dish) for 18 h. The cells were harvested and incubated in Lysis Buffer (50 mM Tris-Cl pH 8.0, 150 mM NaCl, 0.02% Na Azide 0.1 mg/ml PMSF, 1 µg/ml aprotinin, 0.1% NP-40) on ice for 15 min. The mixture was gently mixed by vortexing for 5 min followed by a centrifugation at 12,000 rpm. Supernatant was used for the subsequent analyses. Concentration of protein in the cell lysate was determined with a BCA kit (Pierce, Rockford, IL, USA). For each immunoprecipitation reaction, an aliquot of 1 ml cell lysate was mixed with 10 µl human serum and incubated on ice for 4-6 h followed by the addition of 50 µl of protein A-agarose beads (Santa Crutz Biotechnology, Santa Crutz, CA, USA). The mixture was incubated on ice for 1 h and then centrifuged at 10,000 rpm for 5 min. The pellets were washed three times with 1 ml of NET buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris-Cl pH 7.4, 0.02% Na-azide, 0.01% NP-40). Pellets were resuspended in 25 µl sample buffer and were boiled for 10 min followed by centrifugation as in previous steps. Proteins in the supernatant were separated by SDS-PAGE and visualized by autoradioraphy.

Results

Rat PCNA cDNA was cloned into an *E. coli* expression vector (Fig. 1A). The expression vector was introduced into *E. coli* and expression of the recombinant protein was induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Fig. 1B). Subsequently, the overproduced recombinant protein was isolated and purified by affinity chromatography on a immobilized nickel column (Fig. 1C). The identity and purity of the product after affinity chromatography were demonstrated by SDS-PAGE and Western blot analysis (Fig. 1D).

Purified recombinant PCNA protein was used in ELISA to screen for anti-PCNA autoantibodies in sera of patients with SLE. In the procedure, a constant amount of the recombinant PCNA was coated on each sample well of the ELISA plate. To each sample well, a fixed volume of human serum (1:100 dilution) was added. The reaction mixture was incubated at 37°C for 1 h and the Ag-Ab complexes in the mixture were detected as in Materials and Methods. Results of a typical ELISA screen are shown in Fig. 2. Optical densities above an arbitrary level were considered as positives. The arbitrary level was the sum of mean value plus three standard deviations of normal individuals. By this criterion, we identified 18 positives out of 191 sera. This counts for a frequency of nearly 10%, which is 2-3 times higher than previously reported figures (13, 19). As a comparative study, sera of patients with other autoimmune diseases were similarly screened for anti-PCNA autoantibodies. The results shown in Fig. 2 indicate that only the SLE patients have anti-PCNA autoantibodies.

A previous study using immunoprecipitation procedure has suggested that majority of the anti-PCNA autoantibodies recognizes a conformational epitope (3). Here, taking the advantages of ELISA, we examined if the anti-PCNA autoantibodies could

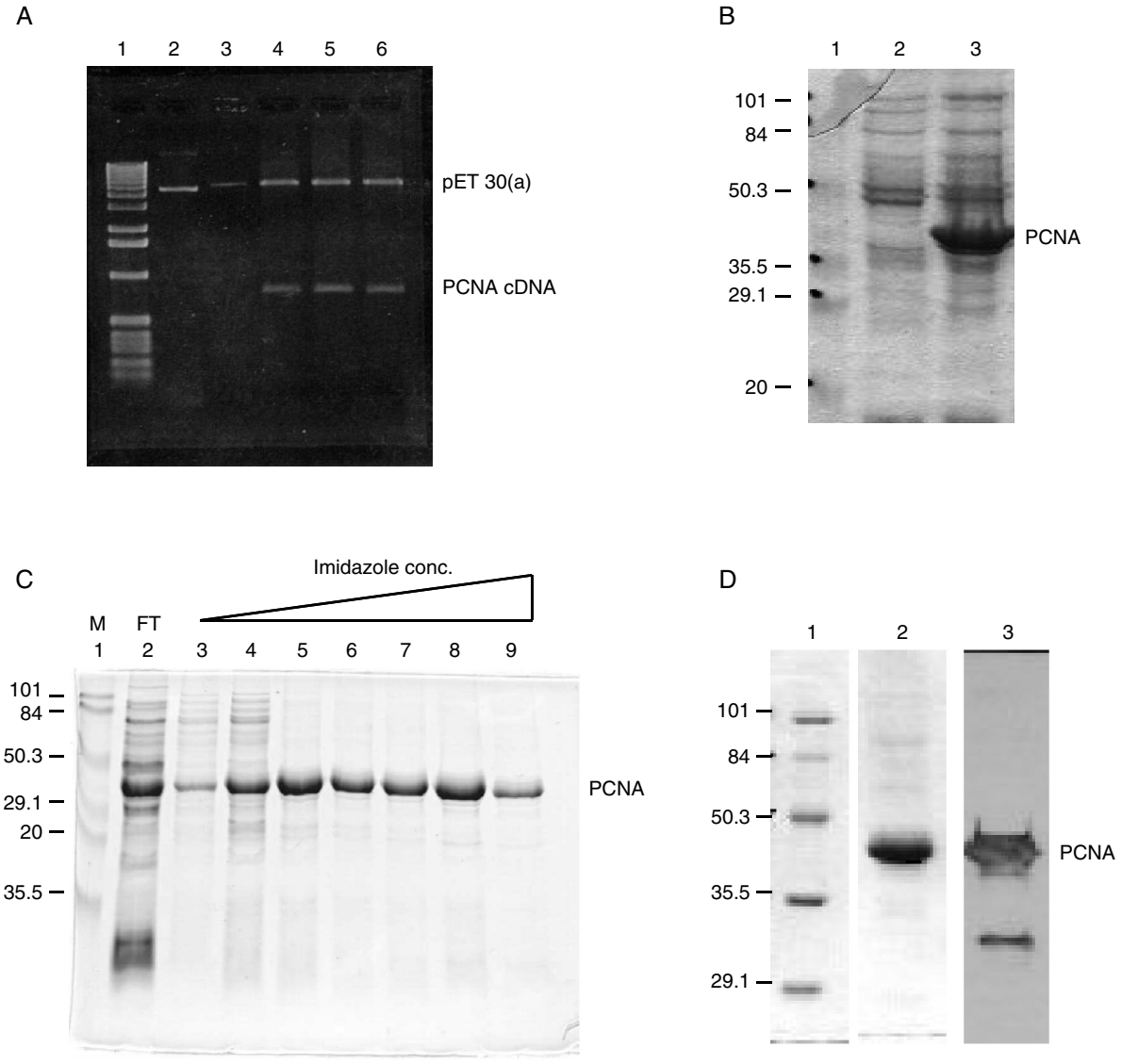

Fig. 1. Construction, expression and purification of PCNA recombinant protein. (A) The PCNA cDNA was digested from PCNA/pET 30(a) plasmid and was separated on a 1% agarose gel. Lane 1 is the 1-kb ladder DNA marker, lane 2 is the undigested plasmid DNA, lanes 3-6 are the various clones of the plasmid. (B) Induction of expression of the recombinant PCNA protein. Lane 1, the molecular weight marker; lanes 2 and 3, cell lysates of non-transformed and transformed cells, respectively. (C) Affinity column purification of recombinant PCNA protein. Lane 1, the marker; lane 2, the flow through; lanes 3-9, eluates obtained with increasing imidazole concentrations. (D) Identification of affinity-purified protein by western blotting. Lane 1, marker; lane 2, Comassie Brilliant Blue staining of purified PCNA; lane 3, Western blotting of PCNA.

differentiate wild-type PCNA from the Y114A mutant. As mentioned earlier, PCNA forms a toroidal structure. The ring structure is critical to the protein activity. The mutant Y114A contains a single substitution at position 114 in which Tyr has been replaced with Ala. The protein fails to form the toroidal structure and is functionally inactive (12). Results of our ELISA study shown (Fig. 3A) that as compared to the wild-type PCNA, the mutant protein was recognized by the autoantibodies with lower efficiencies ranging from 19 to 83%. In contrast, both wild-type and mutant PCNA were equally well recognized by the monoclonal antibody (Ab-1, Oncogene Science, this antibody, also called PC-10, has an epitope located in amino acid sequence 181-195 of PCNA). Five out of 6 antisera were able to differentiate the wild type from the mutant, notably the sample designated as patient 5 which had the highest antibody titer. Consistently, the autoantibodies form precipitin lines only with the wild type but not with mutant Y114A in the immunodiffusion experiment (Fig. 3B).

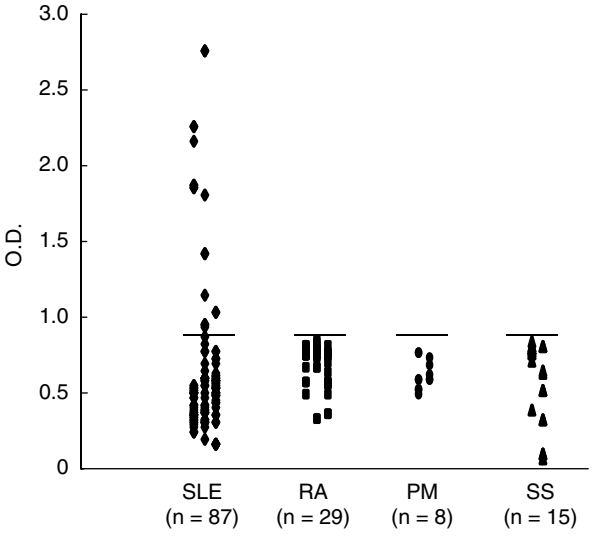

Fig. 2. ELISA screening for anti-PCNA antibodies in patients with different autoimmune diseases. Patient sera of SLE, RA (rheumatoid arthritis), PM (polymyositis) and SS (Sjogren syndrome) were screened for anti-PCNA antibodies by the same ELISA procedure as described above. The patient sera numbers are indicated.

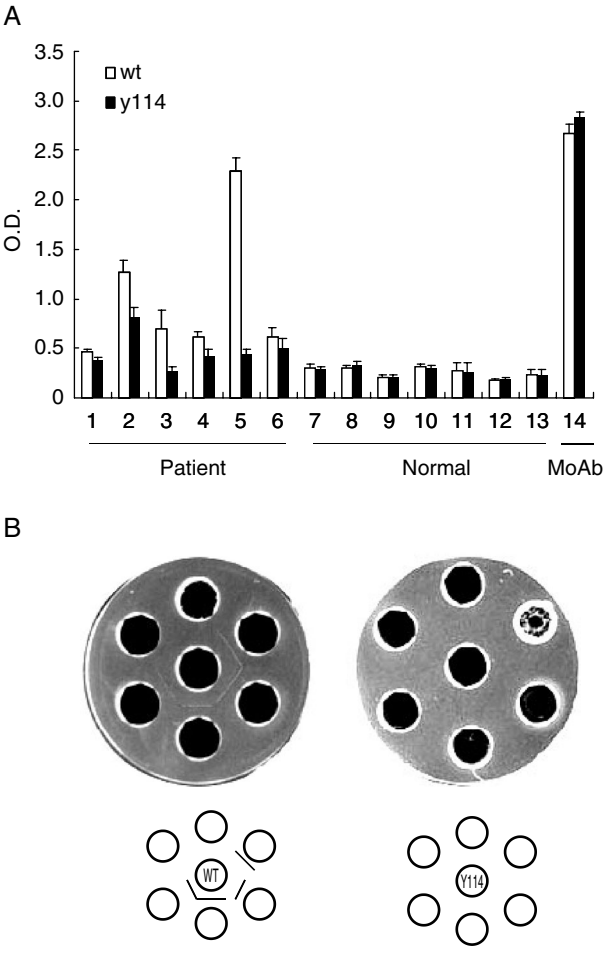

Fig. 3. Autoantibodies preferentially recognize wild-type PCNA. (A) Comparative ELISA using wild-type or mutant Y114A PCNA as antigens. Serum samples 1-6 were SLE sera which were identified as anti-PCNA by in previous screenings. Normal sera and the monoclonal antibody to PCNA (Ab-1, Oncogene) were used as negative and positive controls, respectively. Each sample well was coated with 0.5 μg of wild-type or mutant PCNA. The data were presented as the mean and standard deviations of three independent measurements. Except for serum 6, the SLE serum recognized wild-type PCNA significantly better than the Y114A serum. (B) Comparative immunodiffusion using wild-type or mutant Y114A PCNA as antigens. The double diffusion method was described in Materials and Methods. Wild-type PCNA protein but not Y114A formed precipitins with the autoantibodies.

As mentioned earlier, activity of PCNA is regulated by its association with other components in cells. To investigate the PCNA-associated components, the monospecific autoantibody was used in immunoprecipitation (IP) experiments. The monospecific autoantibody to PCNA was purified from the serum sample of patient 5 by affinity chromatography using immobilized PCNA columns; scarcity and instability of the blood samples prevented us from examining other sera for the IP study. Specificity of the purified autoantibody was verified by both indirect immunofluorescence and immunodiffusion (Fig. 4). In the IP experiments, the monospecific autoantibody was incubated with ^{35}S-labeled crude extracts of Chinese hamster ovary cells (CHO-K1). PCNA-associated proteins were revealed by SDS-PAGE and autoradiography (Fig. 5). In comparing the patterns obtained with control IgG and an monoclonal antibody (lanes 1 and 2, respectively), PCNA and other specific components were 'co-identified' by the monoclonal antibody (lane 2). These specific proteins (indicated by arrows) probably co-precipitated with PCNA by the monoclonal antibody. In contrast, in comparing the patterns obtained with normal human IgG and anti-PCNA autoantibody (lanes 3 and 5, respectively), none of the previous specific proteins were co-precipitated with PCNA by the anti-PCNA autoantibody. In consistent with the observations, the crude anti-serum (containing multi-autoantibodies) did not co-precipitate the specific components either (lane 4). Therefore, the data strongly suggest that the autoantibody recognizes free PCNA but not bound PCNA.

Discussion

In our study, rat PCNA was used as the antigen simply because of the availability of a cDNA clone. Rat and human PCNA proteins are almost identical in

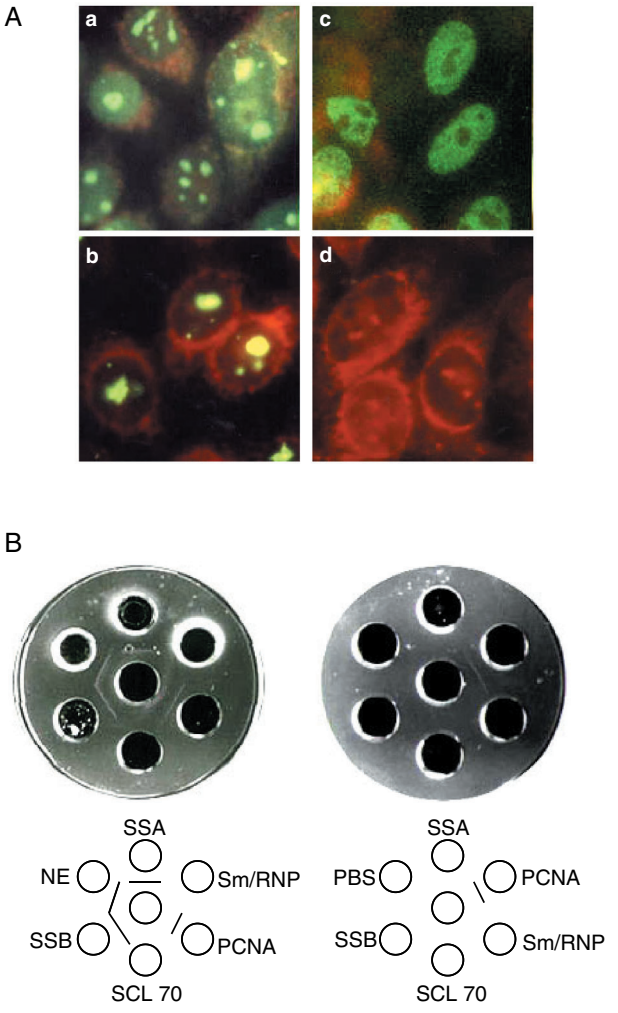

Fig. 4. Purity of the monospecific anti-PCNA autoantibody. (A) Indirect immunofluorescence. SLE sera before purification are shown in a and b; purified anti-PCNA antibodies are shown in c and d. Sera or purified antibody were pre-incubated with free PCNA before use (b and d). (B) Immunodiffusion. SLE sera (top) or purified antibody (bottom) were loaded in the center wells. Antigens SSA, SSB, Sm/RNP, SCL70, PCNA and NE (nuclear extracts) were placed in the surrounding wells.

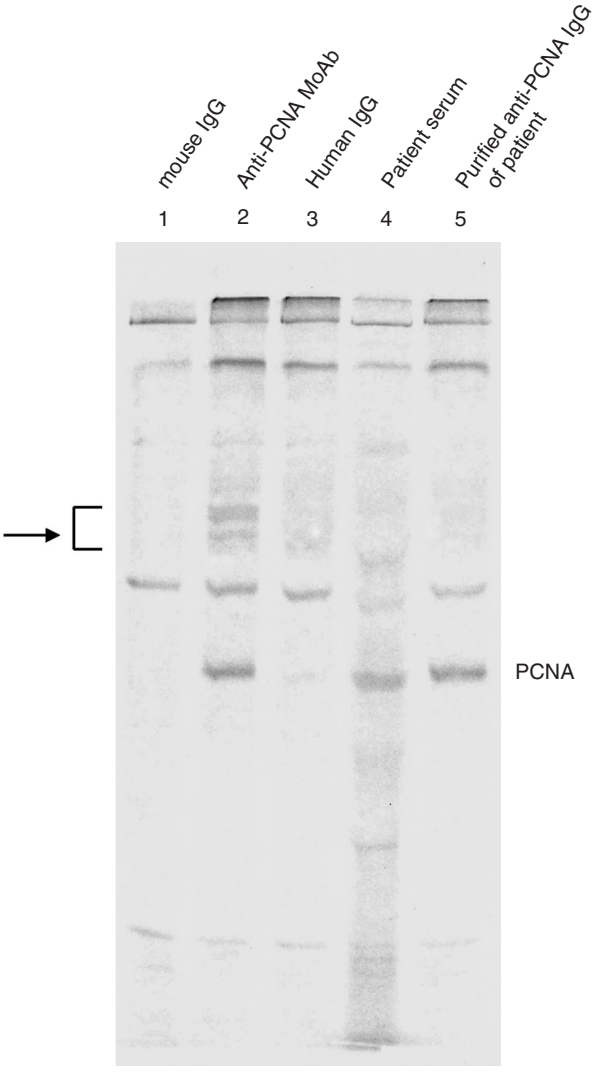

Fig. 5. Monospecific anti-PCNA autoantibody bound PCNA in a free state. Immunoprecipitation was performed as described in Material and Methods. Lane 1, normal mouse IgG (mouse IgG); lane 2, monoclonal antibody to PCNA (Anti-PCNA MoAb; Ab-1, Oncogene); lane 3, normal human IgG (Human IgG); lane 4, patient's antiserum (patient serum); lane 5, purified anti-PCNA autoantibody of patient (Purified anti-PCNA IgG of patient). The band specific to PCNA was indicated and the proteins co-precipitated with PCNA by the monoclonal antibody were marked with arrows.

primary sequences with homology greater than 95%. We found that the frequency of SLE patient sera containing autoantibodies to PCNA was approximately 10%, which is 2-3 times higher than that described previously. The difference is likely due to variations in sensitivity among the different methods used. Despite of such differences, our data agree with previous investigations that autoantibodies to PCNA are only detected in SLE patients as no positive was detected with other autoimmune diseases such as rheumatoid arthritis, polymyositis and Sjogren syndrome (Fig. 2). In characterizing the autoantibodies to PCNA, we found that the majority of antibodies appeared to have better recognition for wild-type PCNA than for mutant Y114A (Fig. 3). In contrast, monoclonal antibody Ab-1/PC-10 did not show any difference between these two proteins. This finding is basically in agreement with the previous report that the bulk of the anti-PCNA autoantibodies recognizes a conformational epitope (3, 27) and supports the native antigen driven theory. The antiserum of patient 5 (Fig. 3) showed a remarkable preference for the wild-type protein (5:1 as compared to mutant

Y114A). The preference suggests that the antibody binds to an active site, or its vicinity, of PCNA.

Previous investigators have found that human antiserum (AK) but not monoclonal antibodies (against PCNA peptides) can neutralize the activity of the auxiliary protein for DNA polymerase δ activity (29). In our study, the autoantibody purified from the antiserum of patient 5 only identifies PCNA, which is not associated with other cellular components. Since PCNA can interact directly with many cellular components, PCNA activity is, thereby, regulated. For instance, $p21^{(Waf1/Cip1)}$ might prevent interactions between PCNA and DNA polymerase δ; $p21^{(Waf1/Cip1)}$ binds to the interloop region of PCNA and may disrupt PCNA trimer (6, 9). Therefore, if the autoantibodies demand a conformational epitope, they would not bind PCNA when the epitope is associated with other protein molecules. Interestingly, a brief report by Takeuchi *et al.* (28) also described that when the anti-PCNA autoantibodies of a patient serum (AK) was immobilized and used as an affinity column, no other components of rabbit thymus extracts were co-purified with PCNA. Hence, it is likely that the immunostaining of PCNA with human antisera (AK or others) in previous studies (4, 5, 23, 30) might represent only a fraction of the total PCNA population. In other words, the PCNAs the epitopes of which were accessible to autoantibody were detected, but those with masked epitopes for some reasons were not. We are currently examining these possibilities to verify whether this has clinical significance.

In summary, we describe herein the use of ELISA to screen anti-PCNA autoantibodies. Perhaps because of the the higher sensitivity of ELISA, a slightly but significant higher frequency than that reported previously was found for SLE patients to have anti-PCNA autoantibodies. The majority of anti-PCNA autoantibodies exhibited preference in recognizing PCNA with a conformational epitope. Furthermore, the conformational epitope-dependent autoantibodies only recognize free PCNA.

Acknowledgments

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References

1. Alan, J. and Robin, T. In: Precipitation Techniques in Agar and Agarose, Immunochemistry in Practice, 1987, pp. 131-147.
2. Bernstein, R., Bunn, C., Hughes, G., Francoeur, A. and Mathews, M. Cellular proteins and RNA antigens in autoimmune disease. *Mol. Biol. Med*. 2: 105-120, 1984.
3. Brand, S., Bernstein, R. and Mathews, M. Autoreactive epitope profiles of the proliferating cell nuclear antigen define two classes of autoantibodies. *J. Immunol.* 153: 3070-3078, 1994.
4. Bravo, R. and Bravo, H.M. Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. *J. Cell. Biol.* 105: 1549-1554, 1987.
5. Celis, J.E. and Celis, A. Cell cycle-dependent variations in the distribution of the nuclear protein cyclin proliferating cell nuclear antigen in cultured cells: subdivision of S phase. *Proc. Natl. Acad. Sci. U.S.A.* 82: 3262-3266, 1985.
6. Chen, I.T., Smith, M.L., O'Connor, P.M. and Fornace, A.J. Jr. Direct interaction of Gadd45 with PCNA and evidence for competitive interaction of Gadd45 and p21Waf1/Cip1 with PCNA. *Oncogene* 11: 1931-1937, 1995.
7. Gramantieri, L., Chieco, P., Giovannini, C., Lacchini, M., Treré, D., Grazi, GL., Venturi, A. and Bolondi, L. GADD45-α expression in cirrhosis and hepatocellular carcinoma: relationship with DNA repair and proliferation. *Hum. Pathol.* 36: 1154-1162, 2005.
8. Gu, B. and Chen, P.L. Expression of PCNA-binding domain of CtIP, a motif required for CtIP localization at DNA replication foci, causes DNA damage and activation of DNA damage checkpoint. *Cell Cycle* 8: 1409-1420, 2009.
9. Gulbis, J.M., Kelman, Z., Hurwitz, J., O'Donnell, M. and Kuriyan, J. Structure of the C-terminal region of $p21^{WAF1/CIP1}$ complexed with human PCNA. *Cell* 87: 297-306, 1996.
10. Huff, J., Roos, C., Peebles, R., Houghten, K., Sullivan, K.F. and Tan, M. Insights into native epitopes of proliferating cell nuclear antigen using recombinant DNA protein products. *J. Exp. Med.* 172: 419-429, 1990.
11. Jaskulski, D., DeRiel, K., Mercer, W., Calabrett, B. and Baserga, R. Inhibition of cellular proliferation by antisense oligodeoxynucleotides to PCNA/cyclin. *Science* 240: 1544-1546, 1988.
12. Jonsson, O., Podust, V., Podust, L. and Hubscher, U. Tyrosine 114 is essential for the trimeric structure and functional activities of human proliferating cell nuclear antigen. *EMBO J.* 14: 5745-5751, 1995.
13. Kaneda, K., Takasaki, Y., Takeuchi, K., Yamada, H., Nawata, M., Matsushita, M., Matsudaira, R., Ikeda, K., Yamanaka, K. and Hashimoto, H. Autoimmune response to proteins of proliferating cell nuclear antigen multiprotein complexes in patients with connective tissue diseases. *J. Rheumatol.* 31: 2142-2150, 2004.
14. Kelman, Z. PCNA: structure, functions and interactions. *Oncogene* 14: 629-640, 1997.
15. Krishna, T., Kong, X., Gary, S., Burgers, P. and Kuriyan, J. Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. *Cell* 79: 1233-1243, 1994.
16. Liu, Y.C., Marraccino, R. and Keng, P. Requirement for proliferating cell nuclear antigen expression during stages of the Chinese hamster ovary cell cycle. *Biochemistry* 28: 2967-2974, 1989.
17. Mathews, M., Bernsstein, R., Franza, B. and Garrels, J. Identity of the proliferating cell nuclear antigen and cyclin. *Nature* 309: 374-376, 1984.
18. Matsuoka, S., Yamaguchi, M. and Matsukage, A. D-type cyclin-binding regions of proliferating cell nuclear antigen. *J. Biol. Chem.* 269: 11030-11036, 1994.
19. Miyachi, K., Fritzler, M. and Tan, E. Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol.* 121: 228-234, 1978.
20. Moriuchi, T., Matsumato, K., Koji, T. and Nakane, P.K. Molecular cloning and nucleotide sequence analysis of rat PCNA/cyclin cDNA. *Nucl. Acids Symp. Ser.* 17: 117-120. 1986.
21. Ogata, K., Ogata, Y., Takasaki, Y. and Tan, E. Epitopes on proliferating cell nuclear antigen recognized by human lupus autoantibody and murine monoclonal antibody. *J. Immunol.* 139: 2942-2946, 1987.
22. Prelich, G., Kostura, M., Marshak, D., Mathews, M. and Stillman, B. The cell cycle regulated proliferating cell nuclear antigen is

required for SV40 DNA replication *in vitro*. *Nature* 326: 471-475, 1987.
23. Robbins, S.A., Vega, de. La. D., Ogata, K., Tan, E.M. and Nakamura, R.M. Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. *Arch. Pathol. Lab. Med.* 111: 841-845, 1987.
24. Sambrook, J., Fritsch, E. and Maniatis, T. Molecular cloning. In: Detection and Analysis of Proteins Expressed from Cloned Genes, Molecular Cloning, 1989, vol. 3, pp. 18.47-18.75.
25. Shivji, M., Kenny, M. and Wood, R. Proliferating cell nuclear antigen is required for DNA excision repair. *Cell* 69: 367-374, 1992.
26. Smith, M., Chen, I. and Bae, I. Interaction of the p53 regulated protein Gadd45 with proliferating cell nuclear antigen. *Science* 266: 1376-1380, 1994.
27. Szüts, D., Christov, C., Kitching, L. and Krude, T. Distinct populations of human PCNA are required for initiation of chromosomal DNA replication and concurrent DNA repair. *Exp. Cell. Res.* 311: 240-250, 2005.
28. Takeuchi, K., Kaneda, K., Kawakami, I., Takasaki, Y. and Hashimoto, H. Autoantibodies recognizing proteins copurified with PCNA in patients with connective tissue disease. *Mol. Biol. Rep.* 23: 243-246, 1996.
29. Tan, C.K., Sullivan, K., Li, X., Tan, E.M., Downey, K.M. and So, A.G. Autoantibody to the proliferating cell nuclear antigen neutralizes the activity of the auxiliary protein for DNA polymerase delta. *Nucl. Acids Res*. 15: 9299-9308, 1987.
30. Toschi, L. and Bravo, R. Changes in cyclin/proliferating cell nuclear antigen distribution during DNA repair synthesis. *J. Cell Biol*. 107: 1623-1628, 1988.
31. Tsai, W., Roos, G., Hugli, T. and Tan, E. Influences of free thiol groups on autoantibody-defined epitopes of proliferating cell nuclear antigen. *J. Immunol*. 149: 2227-2233, 1992.
32. Xiong, Y., Zhang, H. and Beach, D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* 71: 505-514, 1992.