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### Research Article

The chromatin-bound enzyme poly(ADP-ribose) polymerase (ADPRP) is strongly stimulated by DNA with single- or double-stranded breaks, and transfers the ADP-ribose moiety of NAD to nuclear proteins. The activation of ADPRP is important for DNA repair and replication, and also has been postulated to play a role in the pathogenesis of lymphocyte dysfunction associated with chronic inflammatory diseases, and inborn errors of nucleoside metabolism. We have detected high titers of IgG autoantibodies to the ADPRP protein in six patients with rheumatic complaints. No other autoantibodies were detected in any of the six sera. The specificity of the anti-enzyme antibodies was established by (a) immunoprecipitation of ADPRP activity, (b) immunoprecipitation and immunoblotting of both the native 116-kD enzyme and its proteolytic digestion products. ADPRP was purified from human thymus and calf thymus. The autoantibodies reacted equivalently with both enzymes. The anti-ADPRP antibodies had a distinctive immunofluorescent pattern with HEP-2 cells, reacting intensely with nucleoli and metaphase chromosomes, and diffusely with the nucleus. Autoantibodies to ADPRP have not been described previously. The presence of a specific immune response against an enzyme that has been associated with various immunodeficiency syndromes raises intriguing possibilities concerning the relationship between DNA damage, immunodeficiency, and autoimmunity.

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## Human Autoantibodies to Poly(Adenosine Diphosphate–Ribose) Polymerase

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### Abstract

The chromatin-bound enzyme poly(ADP-ribose) polymerase (ADPRP) is strongly stimulated by DNA with single- or double-stranded breaks, and transfers the ADP-ribose moiety of NAD to nuclear proteins. The activation of ADPRP is important for DNA repair and replication, and also has been postulated to play a role in the pathogenesis of lymphocyte dysfunction associated with chronic inflammatory diseases, and inborn errors of nucleoside metabolism. We have detected high titers of IgG autoantibodies to the ADPRP protein in six patients with rheumatic complaints. No other autoantibodies were detected in any of the six sera. The specificity of the anti-enzyme antibodies was established by (a) immunoprecipitation of ADPRP activity, (b) immunoprecipitation and immunoblotting of both the native 116-kD enzyme and its proteolytic digestion products. ADPRP was purified from human thymus and calf thymus. The autoantibodies reacted equivalently with both enzymes. The anti-ADPRP antibodies had a distinctive immunofluorescent pattern with HEp-2 cells, reacting intensely with nucleoli and metaphase chromosomes, and diffusely with the nucleus. Autoantibodies to ADPRP have not been described previously. The presence of a specific immune response against an enzyme that has been associated with various immunodeficiency syndromes raises intriguing possibilities concerning the relationship between DNA damage, immunodeficiency, and autoimmunity.

### Introduction

The nuclear enzyme poly(ADP-ribose) polymerase (ADPRP)<sup>1</sup> requires double-stranded DNA for catalytic activity and is stimulated strongly by DNA containing single- or double-

stranded breaks (1). Using NAD as a substrate, the enzyme adds elongated and branched poly(ADP-ribose) chains to chromatin-bound proteins (2, 3), including the ADPRP enzyme itself (4). Poly(ADP-ribosylation) modulates the interaction between DNA and nuclear proteins (5). The synthesis of poly(ADP-ribose) is thought to play a positive role in the DNA excision-repair process (6, 7), especially at the DNA ligation step (8). However, the excessive activation of ADPRP can also impair cell function and survival by depleting NAD pools (9, 10) and by inactivating nuclear enzymes (11).

ADPRP is found mainly in complex multicellular organisms (12). A major function of the enzyme may be to inactivate and eliminate somatic cells with damaged DNA (7, 13). Recently, the unrestrained formation of poly(ADP-ribose) in lymphocytes has been postulated to be a possible cause of immune dysfunction in patients with inborn errors of adenine nucleoside metabolism, and in chronic inflammatory diseases (14).

DNA-binding proteins are established targets of autoimmunity in patients with systemic autoimmune diseases (15, 16). Considering the possible role of ADPRP in the pathogenesis of immunodeficiency, and the ability of the enzyme to undergo DNA-dependent automodification (4), it was conceivable that ADPRP could represent a potential autoantigen. For these reasons, we systematically searched for autoantibodies to ADPRP in human sera. Here we report our initial findings concerning the presence of high titer, monospecific autoantibodies to ADPRP in a group of patients with rheumatic disorders.

### Methods

**Materials.** All patient sera came from the antinuclear antibody-positive serum bank at the Autoimmune Disease Center of Research Institute of Scripps Clinic. Sera from normal blood donors with antinuclear antibodies were kindly provided by Dr. Marvin Fritzler (University of Calgary, Calgary, Alberta, Canada). Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

**Immunoprecipitation of poly(ADP-ribosyl)ated cellular protein.** Nuclear proteins of the human T lymphoblastoid cell line CCRF-CEM were poly(ADP-ribosyl)ated with [<sup>32</sup>P]NAD (16.3 Ci/mmol sp act, New England Nuclear, Boston, MA) according to the method of Berger et al. (6, 17). Then, cells were lysed in NET-2 buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5 mM EDTA, and 0.5 mM phenylmethyl-sulfonyl fluoride [PMSF]). Solubilized proteins were reacted for 2 h at 4°C with human serum IgG that had been immobilized previously onto protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals, Piscataway, NJ) (18). After centrifugation (10,000 g for 2 min at 4°C) and washing of the beads with NET-2 buffer, antibody-bound protein was solubilized in Laemmli's sample buffer (19) and boiled for 2 min. Immunoprecipitated proteins were fractionated

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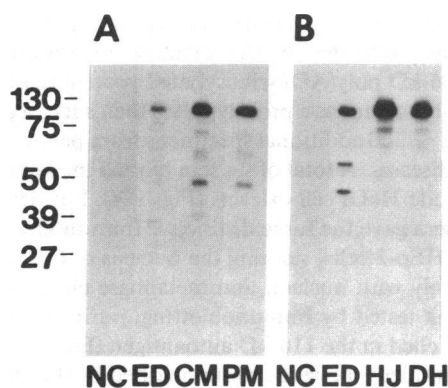
1. *Abbreviation used in this paper:* ADPRP, poly(ADP-ribose) polymerase.

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**Figure 1.** Immunoblotting of autoantibodies to ADPRP. (A) Crude HeLa cell extract and (B) purified nucleoli from transformed human amnion cells were fractionated on a 17.5% polyacrylamide gel containing 0.1% SDS, transferred onto nitrocellulose paper, and immunoblotted with 1:100 diluted sera of a normal control (NC) and patients (E.D., C.M., P.M., H.J., or D.H.). Bound antibody was detected with  $^{125}\text{I}$ -protein A. Molecular weight markers are shown on the left margin as kilodaltons.

on a 12.5% polyacrylamide gel containing 0.1% SDS, and autoradiographed.

*Immunoblotting of HeLa cell extract and purified nucleoli. Con-*

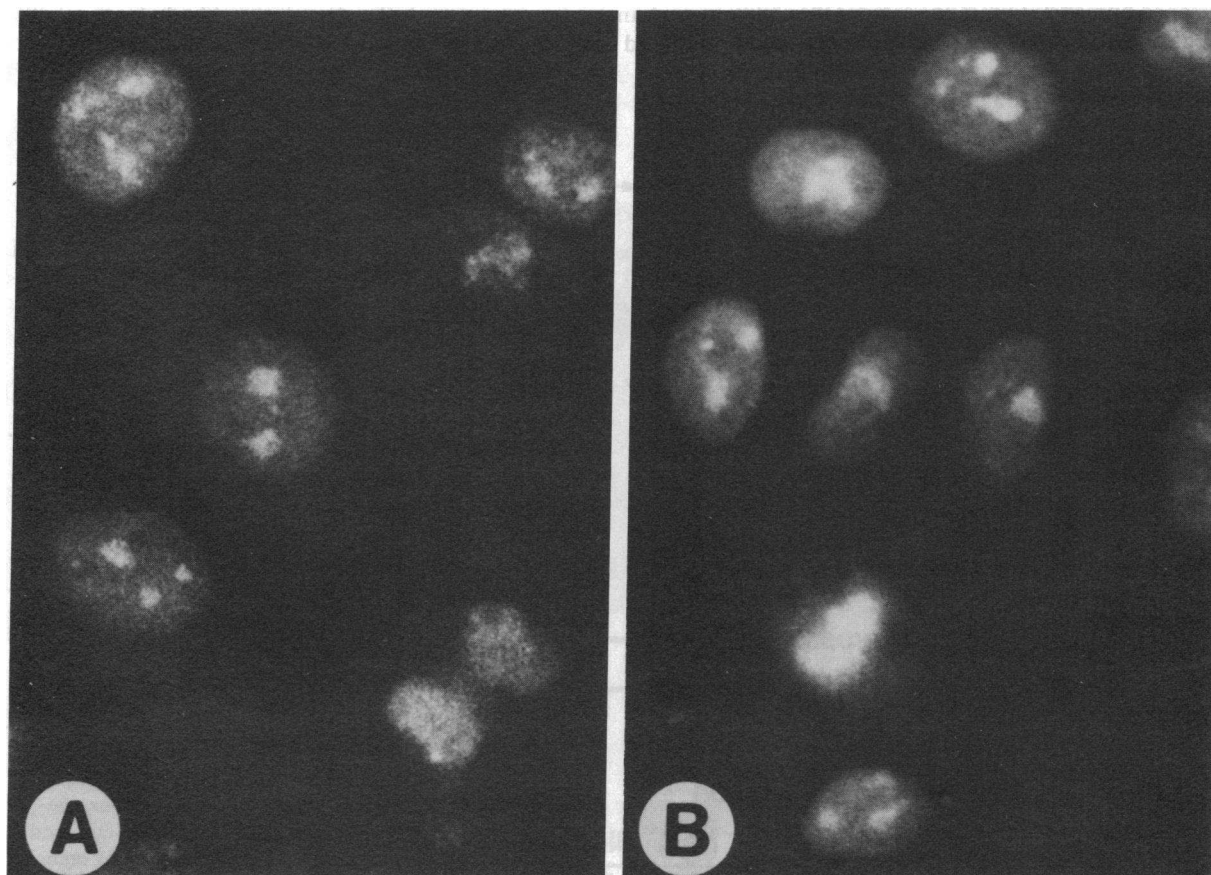
fluent monolayers of HeLa cells were lysed in ice-cold buffer A (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM  $\text{MgCl}_2$ , and 0.5% Nonidet P-40 supplemented with 23 U/ml kallikrein inhibitor). After centrifugation, the supernatant was fractionated on a 17.5% polyacrylamide gel containing 0.1% SDS, and transferred onto nitrocellulose paper. A purified nucleoli fraction was obtained from the nuclear pellet of transformed human amnion cells (20) according to the method of Rothblum et al. (21). The crude HeLa cell extract and the purified nucleoli were immunoblotted with 1:100 diluted sera, and bound antibody was detected using  $^{125}\text{I}$ -protein A (New England Nuclear).

*Immunofluorescent detection of autoantibodies to ADPRP.* Acetone-fixed HEP-2 cells (Bion Enterprises, Ltd., Park Ridge, IL) were reacted with either a serum containing antibodies to ADPRP or affinity purified anti-ADPRP IgG, and followed by fluorescein-conjugated goat anti-human IgG (Tago, Burlingame, CA). Then the cells were examined under a Leitz fluorescent microscope.

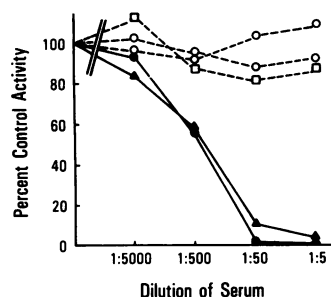
*Purification and analysis of ADPRP.* ADPRP was purified from calf thymus (150 g, Pel-Freeze Biologicals, Rogers, AK) and human thymus (obtained at cardiac surgery) according to the method of Carter et al. (22) through the hydroxyapatite column step.

1- $\mu\text{g}$  aliquots of the purified calf thymus or human thymus ADPRP were treated for 2 h at  $4^\circ\text{C}$  with serial dilutions of antibody-coated Sepharose beads, exactly as described above. After centrifugation, the ADPRP enzyme activities in the antibody-treated supernatants were determined radiochemically (22).

To map the autoantigenic epitopes on ADPRP, 15  $\mu\text{g}$  of the purified calf enzyme was auto-poly(ADP-ribosyl)ated with 0.65  $\mu\text{M}$  [ $^{32}\text{P}$ ]NAD in the presence of 100  $\mu\text{g}/\text{ml}$  calf thymus DNA, as described



**Figure 2.** Immunofluorescent detection of autoantibodies to ADPRP. Acetone-fixed HEP-2 cells were examined by indirect immunofluorescent method using (A) a serum containing antibodies to ADPRP or (B) affinity purified anti-ADPRP IgG, as the first antibody, and fluorescein-conjugated goat anti-human IgG as the second antibody. The diffuse nuclear staining with nucleolar accentuation is apparent.



**Figure 3.** Depletion of ADPRP enzyme activity by autoantibody. 1- $\mu$ g aliquots of purified ADPRP were treated for 2 h at 4°C with serial dilutions of IgG from sera E.D. (●), H.J. (▲), a pair of normal controls (○), and an anti-scl-70 serum (□), each of which had been coated previously onto protein A-Sepharose beads. After centrifugation, the enzyme activities

in the antibody-treated supernatants were determined and expressed as percentages of the antibody-free control (75.0 nmol/min per mg protein).

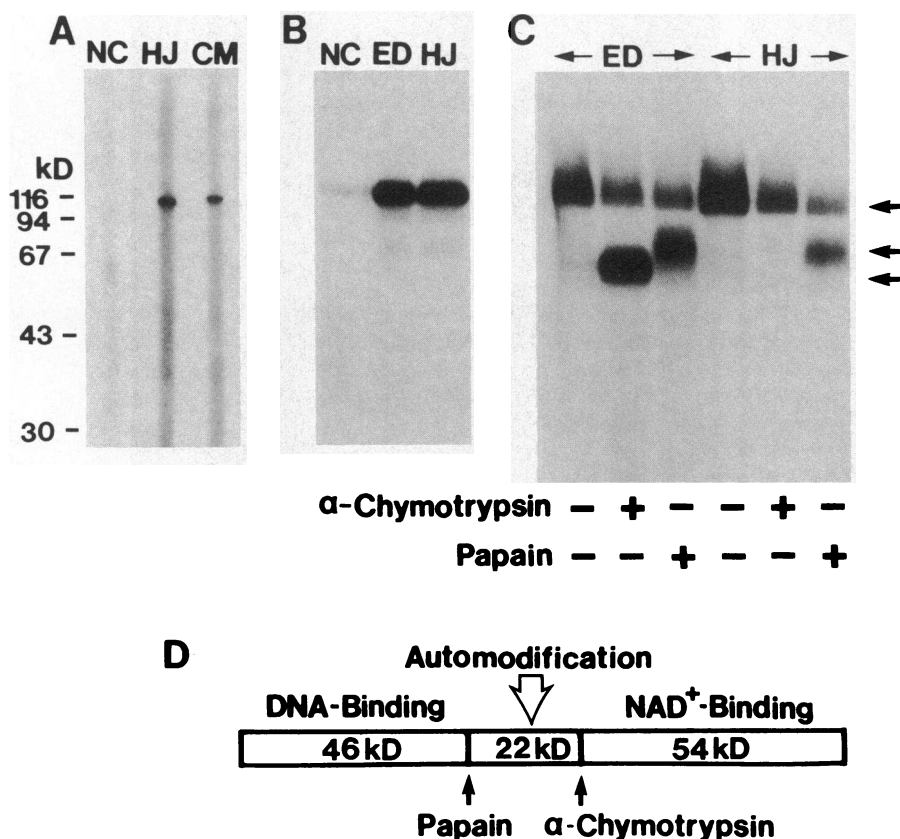
(23). Then, the automodified ADPRP was partially digested either with  $\alpha$ -chymotrypsin (5  $\mu$ g/ml at 25°C for 5 min) or papain-agarose (100  $\mu$ g/ml at 4°C for 5 min). The reactions were terminated by the addition of 1 mM PMSF or by microfiltration, respectively. The proteolytic fragments, or the intact enzyme, were immunoprecipitated with sera containing autoantibodies to ADPRP, as described above.

## Results and Discussion

ADPRP consists of a single 116-kD polypeptide chain with distinct NAD-binding and DNA-binding domains (23). Even in whole cells, ADPRP is the major acceptor of the poly(ADP-ribosylation) reaction (17). Taking advantage of this characteristic, we screened human autoimmune sera for the ability to immunoprecipitate [ $^{32}$ P]NAD-labeled proteins from human

CEM T lymphoblasts. Among 21 initial antinuclear antibody-positive sera that were examined by this method, one immunoprecipitated a 116-kD poly(ADP-ribosyl)ated protein (data not shown). Using this serum as a prototype, we then surveyed by immunoblotting ~ 200 additional specimens from patients with autoimmune diseases. A total of six sera blotted the same 116-kD band in crude HeLa cell extracts (Fig. 1 A). Furthermore, each of the sera gave the same distinctive immunofluorescent pattern on HEP-2 cells, staining the nucleus diffusely, and reacting intensely with nucleoli and metaphase chromosomes (Fig. 2 A). As tested by immunoblotting, purified nucleoli were also enriched in the 116-kD autoantigen (Fig. 1 B). The smaller polypeptides that were immunoblotted by the antibodies in Fig. 1 were shown to be degradation fragments of the 116-kD protein, because antibodies eluted from each of the smaller bands reacted with the 116-kD band (data not shown).

To prove that the six sera specifically recognized the ADPRP protein, the enzyme was purified 3,800-fold from calf thymus, and 1,000-fold from human thymus. During the purification steps, autoantigenic activity of ADPRP was determined by enzyme-linked immunosorbent assay (ELISA) (24). ADPRP activity and the autoantigenic activity co-eluted from both a DNA-cellulose column and a hydroxyapatite column (results not shown). As shown in Fig. 3, incubation of the purified calf thymus ADPRP with patients' IgG, coupled to protein A-Sepharose, depleted the ADPRP activity in a dose-dependent manner. In control experiments, incubation of the enzyme with protein A-Sepharose beads coated with normal human serum or a control antinuclear antibody (anti-scl 70) did not alter the ADPRP activity. The purified enzyme yielded



**Figure 4.** Immunoblotting and immunoprecipitation of purified ADPRP. (A) Purified ADPRP from calf thymus was fractionated by SDS-12.5% polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, and immunoblotted with a 1:100 dilution of serum from a normal control (NC), or from patients H.J. and C.M. (B) 30  $\mu$ g ADPRP was auto-poly(ADP-ribosyl)ated with 0.65  $\mu$ M [ $^{32}$ P]-NAD. Radiolabeled ADPRP was immunoprecipitated with IgG from a normal control (NC), or from patients E.D. and H.J., and was fractionated by SDS-12.5% polyacrylamide gel electrophoresis and autoradiographed. (C) 15  $\mu$ g ADPRP was auto-modified with [ $^{32}$ P]NAD, and then was partially proteolyzed either with  $\alpha$ -chymotrypsin (5  $\mu$ g/ml at 25°C for 5 min) or papain-agarose (100  $\mu$ g/ml, 4°C, 5 min). After the termination of the reaction, the proteolytic fragments were immunoprecipitated either with the serum of patient E.D. or H.J. The arrows on the right margin of the figure show the native enzyme, the NAD-binding domain plus the automodification site, and the DNA-binding domain plus the automodification site, respectively, from the top. (D) The proposed components of the ADPRP polypeptide, according to Kameshita et al. (23).

Table I. Clinical Manifestations of Patients with Anti-ADPRP Antibodies

Patient no.	Age/sex	Presenting symptoms	Other signs and symptoms	Clinical diagnosis
1	44/F	Leg paresthesias	Parotid mass, dry eyes, dry mouth Elevated ESR (44 mm/h)	Sjögren's syndrome Sensory polyneuropathy
2	58/F	Myalgia with muscle weakness	Abnormal liver function tests Schirmer's test (5 mm) Elevated ESR (76 mm/h)	Possible Sjögren's syndrome Chronic liver disease
3	62/F	Myalgia + fatigue		Sensory polyneuropathy
4	65/M	Diffuse leg pain	Elevated ESR (48 mm/h) Mass on chest x-ray	Bronchogenic carcinoma

ESR, Erythrocyte sedimentation rate (Westergren method).

the same immunoblotting pattern as crude cell extracts (Fig. 4 A). Similar results were obtained with the human enzyme.

Affinity chromatography was used to isolate the anti-ADPRP autoantibodies from the serum of one patient. Purified calf thymus ADPRP was coupled to Sepharose and incubated with patient E.D.'s serum. After extensive washing, the bound autoantibody was eluted with 0.1 M glycine-HCl, pH 2.5, and neutralized. The affinity purified autoantibody gave the same immunofluorescent pattern on HEp-2 cells as whole serum (Fig. 2 B), and also recognized the 116-kD ADPRP protein on immunoblots.

To localize the autoantigenic epitopes on ADPRP, the purified calf enzyme was automodified with [ $^{32}$ P]NAD, partially proteolyzed, and then analyzed by immunoprecipitation. Previous experiments have shown that digestion of ADPRP with papain yields a 74-kD fragment that contains the NAD-binding and automodification domains (23). In contrast, digestion with  $\alpha$ -chymotrypsin produces a 66-kD fragment that includes the DNA-binding and automodification domains (Fig. 4 D, reference 23). Besides reacting with the intact enzyme, two of the six sera immunoprecipitated both the 74- and 66-kD fragments; the other four sera recognized the 74-kD fragment, but not the 66-kD fragment (Fig. 4 C). These results indicate that the autoantigenic epitopes on ADPRP reside in both the DNA-binding and the NAD-binding domains. However, the failure of four sera to react with the poly(ADP-ribosyl)ated 66-kD fragment shows that they do not contain precipitating antibodies to poly(ADP-ribose), which have been reported in lupus erythematosus (25). Consistent with this result, the sera also did not precipitate poly(ADP-ribosyl)ated histones. Nor did they contain autoantibodies against DNA, histone, n-ribonucleoprotein, Sm, SS-A(Ro), SS-B(La), Ku, or scl-70 (topoisomerase I), as measured by standard immunofluorescence, immunodiffusion, or ELISA methods (15, 26, 27). Remarkably, each serum reacted monospecifically with ADPRP. The anti-enzyme antibodies were predominantly IgG, and contained both kappa and lambda light chains.

Clinical data on four of the patients with anti-ADPRP autoantibodies were available, and are summarized in Table I. Three patients complained of persistent neuromuscular pain, principally affecting the lower extremities, and two were considered to have a sensory polyneuropathy. One patient had classical Sjögren's syndrome. The patients with autoantibodies against ADPRP did not have any symptoms or signs of systemic lupus erythematosus, scleroderma, or rheumatoid ar-

thritis. Furthermore, > 100 sera from patients with systemic lupus erythematosus or scleroderma, that were tested by immunoblotting or ELISA, did not have anti-ADPRP antibodies. Finally, an initial survey of antinuclear antibody-positive sera from healthy blood donors failed to reveal any specimen with antibody against ADPRP.

For unknown reasons, autoantibodies against DNA-binding proteins are common in rheumatic diseases (15). Therefore, it is especially noteworthy that in the patient group reported here, the autoimmune response was directed exclusively against the ADPRP enzyme. ADPRP is the principal acceptor of poly(ADP-ribose) in cells with massive DNA strand breaks (17). Excessive activation of ADPRP in lymphocytes can impair immune responses (13, 14). In preliminary experiments, we have not detected any difference between the numbers of DNA breaks in the peripheral blood lymphocytes in a patient with autoantibodies against ADPRP and in normal subjects. Moreover, patients with generalized DNA repair defects have not been shown to have an increased incidence of antinuclear antibodies. However, it is still conceivable that the accumulation of DNA strand breaks in a minor population of antigen-presenting cells, whether caused by an exogenous agent or by internal metabolic changes, could lead to the modification of ADPRP. In this regard, the poly(ADP-ribose) polymer is known to be immunogenic (28), and chemically modified proteins often demonstrate increased immunogenicity. Perhaps these factors interact to promote the synthesis of autoantibodies against the ADPRP enzyme.

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