Serological Cross-reactivity between a Human Ro/SS-A Autoantigen (Calreticulin) and the λRAL-1 Antigen of *Onchocerca volvulus*

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Abstract

We have cloned and sequenced a 46-kD Ro/SS-A autoantigen gene that is the human homologue of the calcium-binding protein, calreticulin. The sequence of this 46-kD Ro/SS-A protein (calreticulin) has significant homology to λRal-1, a recombinant cDNA clone corresponding to a major antigen of the nematode, *Onchocerca volvulus*, the infectious agent of onchocerciasis. We therefore sought to determine whether antibodies produced by onchocerciasis patients might crossreact with the human 46-kD Ro/SS-A autoantigen (calreticulin). 20 of 22 sera from Liberian onchocerciasis patients who had no known evidence of autoimmune disease were found to contain antibodies that reacted with the 46-kD Ro/SS-A (calreticulin) by immunoblot analysis. Characteristic of sera reactive with Ro/SS-A antigens, some onchocerciasis sera also immunoprecipitated the Ro/SS-A-associated hY RNAs. In addition, a monoclonal antibody raised against *O. volvulus* organisms reacted to purified human WiL-2 cell 46 kD Ro/SS-A antigen (calreticulin) by ELISA. These results strongly suggest that onchocerciasis patients produce antibodies that crossreact with the 46-kD human Ro/SS-A autoantigen (calreticulin) and raise the possibility that infectious organisms such as *O. volvulus* might play a triggering or exacerbating role in the human Ro/SS-A autoimmune response. (*J. Clin. Invest.* 1992. 89:1945–1951.) Key words: ribonucleoprotein • autoimmunity • endoplasmic reticulum • calcium-binding protein • onchocerciasis

Introduction

Autoantibodies to the Ro/SS-A ribonucleoproteins have been associated with several autoimmune diseases, including primary Sjögren's syndrome (SS),[1] subacute cutaneous lupus erythematosus (SCLE), neonatal lupus erythematosus, antinuclear antibody negative systemic lupus erythematosus (SLE), and systemic lupus erythematosus-like disease secondary to homozygous C2 or C4 complement deficiency (1–6). A considerable body of evidence now suggests that there are at least four immunologically distinct Ro/SS-A molecules: 2 of 60 kD mol mass, as well as 52-kD and 54-kD proteins (7–10). A novel class of small RNAs, the hY RNAs, is associated, either directly or indirectly, with members of the Ro/SS-A protein family. However, the functions of the Ro/SS-A proteins and hY RNAs remain elusive (11–13).

Our laboratory recently isolated a cDNA clone corresponding to one of the Ro/SS-A proteins (13). The mature polypeptide encoded by this gene has a calculated molecular mass of 46 kD, but migrates aberrantly at 60 kD in SDS-PAGE. Other workers examining this protein as a calcium-binding protein have reported that it has an apparent molecular mass in SDS-PAGE as low as 52 kD. Other proteins having a negative charge similar to this protein have been shown to have equally high anomolous molecular masses due to a gel migration artifact (13). The amino acid sequence of this 46-kD Ro/SS-A protein bears no resemblance to those of other Ro/SS-A polypeptides that in SDS-PAGE have molecular mass of 60 (14) and 52 kD (10). The 46-kD Ro/SS-A gene, which is found as a single copy on the short arm of chromosome 19, is highly conserved among several mammalian species and appears to be identical to human calreticulin, a high-affinity calcium binding protein normally associated with the endoplasmic reticulum (15). This protein is also quite similar to the neuronal protein 407 of the snail *Aplysia californica* and to a protein of *Drosophila melanogaster* (15). Our laboratory has recently confirmed that the native WiL-2 46-kD Ro/SS-A protein, like calreticulin, binds calcium.[2]

Computer homology searches have revealed that human and rat calreticulin have significant (63 and 64%, respectively) amino acid sequence homologies to the λRal-1 cDNA clone isolated from an *Onchocerca volvulus* expression cDNA library (Fig. 1) (13, 16). A fusion protein expressed by the λRal-1 clone reacted strongly with an antiserum derived by immunization with the infectious form of this nematode, the third stage larva, or L3. Immunoaffinity purified anti-λRal-1 antibodies bound a protein from adult *O. volvulus* extracts that had an apparent molecular mass of 46 kD, though the abbreviated open reading frame of the λRal-1 cDNA clone itself coded for a 39.1-kD protein (17).

Because of the sequence homology between the 46-kD Ro/

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1. *Abbreviations used in this paper:* ANA, antinuclear antibody; CIE, counterimmunoelectrophoresis; RF, rheumatoid factor; SCLE, subacute cutaneous lupus erythematosus; SS, Sjögren's syndrome.

2. Lieu, T. S., D. P. McCauliffe, P. Volpe, B. H. Alderson-Lang, C. Heilmann, E. Leberer, M. Michalak, L. Fliegel, J. D. Capra, and R. D. Sontheimer. 1992. Further evidence supporting the identity of a native human Wil-2 cell 46 kD Ro/SS-A autoantigen with the highly-conserved calcium-binding protein, calreticulin (manuscript submitted for publication).

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Hu - M L L S V P L L L G L L G L A V A E P A V Y F K E Q F L D G D G W T S R  36

Hu - W I E S K H K S D F G K F V L S S G K F Y G D E E K D K G L Q T S Q D A  72
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On - - - - - - - - - incomplete 5' end - - F Y G D A V K D K G L K T T Q D A

Hu - R F Y A L S A S F E - P F S N K G Q T L V V Q F T V K H E Q N I D C G G  107
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On - K F Y S I C A K F D K S F S N K G K S L V I Q F S V K H E Q D I D C G G

Hu - G Y V K L F P N S L D Q T D M H G D S E Y N I M F G P D I C G P G T K K  143
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On - G Y V K L M A S D V N L E D S H G E T P Y H I M F G P D I C G P G T K K

Hu - V H V I F N Y K G K N V L I N K D I R C K D D E F T H L Y T L I V R P D  179
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On - V H V I F H Y K D R N H M I K K D I R C K D D V F T H I Y T L I V N S D

Hu - N T Y E V K I D N S Q V E S G S L E D D W D F L P P K K I K D P D A S K  215
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On - N T Y F V Q I D G F K A E S G E L E A D W D F L P P K K I K D P D A K K

Hu - P E D W D E R A K I D D P T D S K P E D W D K P E H I P D P D A K K P E  251
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On - P E D W D E R E F I D D E D D K K P E D W D K P E H I P D P D A K K P E

Hu - D W D E E M D G E W E P P V I Q N P E Y K G E W K P R Q I D N P D Y K G  287
     : : : : : : : : : : : : :       : : : : : : : : : :   :     : :   : : :
On - D W D D E M D G E W E P P M V D N P E Y K G E W K P K Q K K N P A Y K G

Hu - T W I H P E I D N P E Y S P D P S I Y A Y D N F G V L G L D L W Q V K S  323
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On - K W I H P E I E I P D Y T P D D N L Y V Y D D I G A I G F D L W Q V K S

Hu - G T I F D N F L I T N D E A Y A E E F G N E T W G V T K A A E K Q M K D  359
     : : : : :         :           :     : :     :       :       : :     :
On - G T I F D D V I V T D S V E E A K K F G E K T L K I T R E G E K K - K G

Hu - K Q D E E Q R L K E E E E D K K R K E E E E A E D K E D D E D K D E D E  395
     :         :     :   :       :     : :                         :
On - K K T K K Q K - K K E K N E K I K K E K M K K R K R A N R K K K K *

Hu - E D E E D K E E D E E E D V P G Q A K D E L                              417
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Figure 1. The 46-kD Ro/SS-A protein (calreticulin) amino acid sequence (Hu) compared with that for the λRal-1 protein (On).

SS-A protein (calreticulin) and the *O. volvulus* λRal-1 antigen, we questioned whether onchocerciasis patients might develop an immune response that crossreacts with the 46-kD Ro/SS-A antigen (calreticulin). In the present study, we examined the reactivity of sera from Liberian onchocerciasis patients toward the native 46-kD Ro/SS-A antigen purified from the human WiL-2 cell line, as well as to fragments of this protein derived by partial digestion with staphylococcal V8 protease. The results of these studies suggest that onchocerciasis patients produce antibodies that crossreact with a 46-kD human Ro/SS-A autoantigen, and support the possibility that infectious organisms that express a highly conserved gene product similar to calreticulin may play a role in triggering or exacerbating a Ro/SS-A antigen-directed autoimmune response.

Methods

Antigen source. Ro/SS-A antigen was isolated from extracts of an EBV-transformed human B lymphoblastoid cell line (WiL-2) by a procedure that was previously described (18, 19). Briefly, cells were grown in Eagle's medium supplemented with 2 mM glutamine, sodium pyruvate, nonessential amino acids, 10% fetal calf serum, penicillin 10,000 μ/ml, and streptomycin (10 mg/ml). The cells were centrifuged at 35× *g* for 12 min and washed with PBS three times. The packed cells (40 ml) were then mixed with the same volume of PBS containing 1 mM PMSF. The suspension was sonicated on ice with 10 15-s pulses using a sonicator (Heat Systems-Ultrasonics, Inc., Farmingdale, NY) at a setting of 9. The sonicate was then centrifuged at 12,100× *g* for 1 h and the supernatant subjected to ammonium sulfate precipitation. The 33–60% ammonium sulfate fractions were applied to a polybuffer ion exchange column and eluted with a stepwise NaCl gradient. The 0.5–1.0 M NaCl fractions were further purified by preparative native PAGE. Ro/SS-A antigenic activity was then eluted from gel slices. The 260/280 absorbance ratio suggests that calreticulin is still associated with nucleic acid at this stage of purification. The presence of Ro/SS-A antigenic activity was monitored throughout this purification procedure by counterimmunoelectrophoresis (CIE) against monospecific anti-Ro/SS-A sera.

Human antisera. Onchocerciasis sera were obtained from patients in Liberia with localized onchocerciasis (chronic hyperreactive onchodermatitis) or generalized onchocerciasis, diagnosed either by evaluation of skin snips or a Mazzotti reaction with diethylcarbamazine (DEC) (20). These patients had no overt manifestations of SLE or other autoimmune disease. African control sera obtained from persons living in an area hypoendemic for onchocerciasis in western Liberia, who were without subcutaneous nodules, demonstrated a negative Mazzotti reaction to DEC, and lacked microfilaria in two skin snips (collagenase technique [20]). United States control sera were obtained from unselected healthy subjects. Anti-Ro/SS-A sera were selected by the presence of a single precipitin line by Ouchterlony analysis, which demonstrated complete identity with prototypic sera used in previous studies (18, 19). In a double immunodiffusion assay, the sera showed a line of identity with the standard monospecific anti-Ro/SS-A reference serum (AF/CDC7) from the CDC in Atlanta, GA. In addition, the anti-Ro/SS-A sera immunoprecipitated the four hY RNAs using ^{32}P-labeled WiL-2 extract.

Monoclonal antibody to O. volvulus. A panel of murine monoclonal antibodies were produced by immunizing Balb/c mice with homogenates of female *O. volvulus.* (21). 13 of these antibodies were tested in ELISA against purified native human WiL-2 cell 46-kD Ro/SS-A antigen (calreticulin) and ovalbumin as a negative control. The results were compared with control hybridoma culture media. The following adaptation of a previously described ELISA technique was used (18). Purified native 46-kD Ro/SS-A antigen (calreticulin) (5 μg/ml) in PBS was added to wells of an Immulon II microtiter plate treated with 0.5% glutaraldehyde and incubated for 16 h at 4°C. The plates were washed with PBS-Tween, and the remaining sites were coated with 1% ovalbumin for 1 h. After washing three times with PBS-Tween, test sera diluted 1:100 with 1% BSA and 0.5% bovine gamma globulin in PBS-Tween were added and incubated for 2 h. The plates were then washed three times with PBS-Tween. Biotin-conjugated goat anti-mouse Ig (1:4,000) was then added to the plates and incubated for 2 h at room temperature. The plates were washed with PBS-Tween three times at 15-min intervals. Peroxidase-conjugated avidin (1:4,000) was then added and incubated for 2 h. The plates were washed with PBS-Tween in a similar manner. The color was developed by adding a peroxidase substrate solution containing 1 mg/ml of 2,2′-azino-di-(3-ethyl-benzthiazoline-6-sulfonic) acid and 0.005% H_2O_2 in McIlvaine's buffer, pH 4.6. The optical density was measured with a Titertek Multiskan. Control ELISA plates were prepared by substituting 1% ovalbumin for the purified Ro/SS-A antigen (calreticulin).

SDS-PAGE and immunoblot analysis. The WiL-2 cell-derived proteins were separated using a 12.5% polyacrylamide gel in the presence of SDS (0.1%), according to the method of Laemmli (22), and immunoblot analysis were performed as described by Towbin et al. (23). Horseradish peroxidase-conjugated goat anti-human IgG followed by diaminobenzidine tetrahydrochloride (12.5 mg/100 ml Tris buffer, pH 7.6) was used to detect the binding of sera to the immunoblot. *Staphylococcus aureus* V8 protease (Sigma Chemical Co., St. Louis, MO) was used for protein cleavage, according to established protocols (24).

Immunoprecipitation of radiolabeled cell lysates. 8×10^6 WiL-2 cells were labeled with 12.5 mCi [^{32}P]orthophosphoric acid (carrier free; New England Nuclear, Boston, MA) in low phosphate minimal essential media (Gibco Laboratories, Grand Island, NY) with 10% fetal calf serum at 37°C for 16 h. Cells were harvested, washed three times in cold wash buffer (NaCl 150 mM and Tris 50 mM, pH 7.4), resuspended in 10 cm^3 NET-2 buffer (NaCl 150 mM, Tris 50 mM, pH 7.4, and NP-40 0.05%), and sonicated three times (40 s each) at setting 4

using a pulsed sonicator (Branson Ultrasonics Corp., Danbury, CT). The lysates were cleared by centrifugation, 25 U RNasin (Promega Corp., Madison, WI)/ml was added, and supernatant was used immediately for immunoprecipitation.

80 μl antisera was added to 102 μl protein-A Sepharose 50% (Zymed Labs., Inc., San Francisco, CA), incubated 0.5 h with rocking at 4°C, washed three times with NET-2 buffer, and added to 0.5 cm^3 of the ^{32}P-labeled supernatant. This was incubated with rocking at 4°C for 1 h and washed three times with NET-2. The nucleic acids were then extracted with phenol/chloroform, precipitated with ethanol, and run on 10% polyacrylamide-7 M urea gels at 400 V.

Synthetic oligonucleotide construction. A 24 mer oligonucleotide corresponding to hY3 RNA was synthesized using a DNA synthesizer (model 380B; Applied Biosystems Inc., Foster City, CA).

Northern filter hybridization. RNA extracted from immunoprecipitants of nonradiolabeled WiL-2 cell lysates was electrophoresed in a 1.2% agarose-formaldehyde gel, electrophoretically transferred to a Zeta-Probe nylon-reinforced support membrane, according to the manufacturer's guidelines (Bio-Rad Laboratories, Richmond, CA), hybridized with a radiolabeled 24-base oligonucleotide complementary to hY3 RNA, and washed at 73.5°C in tetramethylammonium chloride buffer by an established protocol (25).

Serial immunoprecipitation of radiolabeled cell lysates. Sequential adsorption of radiolabeled WiL-2 lysates was performed in a manner similar to that described above, with the exception that 100 μl of sera with 150 μl of protein-A Sepharose 50% was used in each incubation step, and the supernatants of the immunoprecipitations were used for subsequent immunoprecipitations.

Results

Standard autoimmune serology. Immunodiffusion and counterimmunoelectrophoresis studies against WiL-2 cell extracts failed to demonstrate precipitation using the onchocerciasis patient sera, despite clear precipitation lines with monospecific positive control anti-Ro/SS-A sera (data not shown). The absence of precipitin lines using onchocerciasis patient sera also indicates the lack of other autoantibody specificities such as U1 RNP, Sm, and La/SS-B. Analysis of 24 randomly selected onchocerciasis patient sera were examined for rheumatoid factor (RF), antinuclear antibody (ANA) (HEp-2 cell substrate), and anti-double stranded DNA antibody (*Crithidia luciliae* substrate). None produced positive reactions in any of these assays.

Immunoblot of WiL-2 cell extracts. WiL-2 cell extracts were subjected to partial digestion with staphylococcal V8 protease before electrophoresis and immunoblotting. Under the conditions used, this step yields two peptides of 37 kD (the carboxyl-terminal fragment) and 23 kD (the amino-terminal fragment), in addition to the intact undigested 60-kD protein and a minor fragment of 19 kD (Fig. 2). Immunoblotting with anti-Ro/SS-A sera from patients with SCLE yielded bands corresponding to 60, 23, and 19 kD.

20 of 22 onchocerciasis sera were positive when tested by immunoblot. Of these, 3 (14%) bound to all three major bands, 2 (9%) bound to the 60- and 37-kD bands, 2 others (9%) to the 60- and 23 bands, and 12 (55%) to the 60-kD band alone. One serum bound only the 23-kD band and two demonstrated no immunoreactivity with these proteins. No significant difference in binding specificities was noted between the sera of generalized and localized onchocerciasis patients. These heterogeneous findings are in contrast to those seen with SCLE sera that have consistently reacted with only the 60-, 23-, and 19-kD fragments (26). Of the five African control sera examined by immunoblot, only one was weakly reactive with the 60-kD band. The five U. S. control sera examined by immunoblot all failed to react with the intact or digested 46-kD Ro/SS-A (calreticulin) bands.

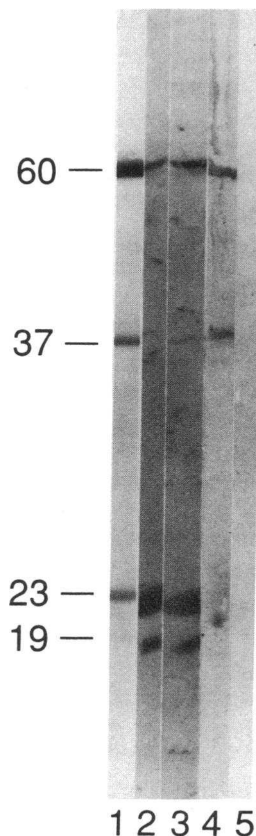

Figure 2. Immunoblot of purified human 46-kD Ro/SS-A antigen (calreticulin) after limited staphylococcal V8 protease digestion. (Lane *1*) An amido black stain of the resultant Ro/SS-A protein fragments after SDS-PAGE. (Lanes *2* and *3*) Immunoblots using sera from two SCLE patients. (Lane *4*) Serum from a patient with generalized onchocerciasis. (Lane *5*) Serum from a normal subject from the U. S.

Crossreactivity of O. volvulus monoclonal antibodies. 1 of 13 *O. volvulus*-reactive murine monoclonal antibodies (SNII C6) reacted significantly with the human WiL-2 cell 46-kD Ro/SS-A autoantigen (calreticulin) by ELISA (Table I). The ELISA optical density produced by this antibody was greater than two standard deviations above the mean of that produced by control culture medium. This antibody was not reactive with ovalbumin, a similarly charged negative control protein. This monoclonal antibody also reacted with a 20-kD *O. volvulus* protein in Western blot analysis (data not shown). This could represent a protease digestion fragment of the *O. volvulus* equivalent of calreticulin.

Immunoprecipitation of radiolabeled hY RNAs. Anti-Ro/SS-A antibodies have been defined in part by their ability to immunoprecipitate four small RNAs, hY1-hY5 (hY2 is a degraded form of hY1), from HeLa cells (4, 11). Therefore, to further confirm that the protein recognized by the onchocerciasis sera was indeed a Ro/SS-A autoantigen, we performed electrophoresis of the nucleic acids from immunoprecipitates derived from WiL-2 cells labeled in vivo with [^{32}P]orthophosphate. Precipitation of hY RNAs was seen using both SCLE patient sera and sera from onchocerciasis patients (Fig. 3 *A*). While only hY1-hY5 were precipitated by the SCLE sera, a more variable pattern was seen with the onchocerciasis sera. For example, bands corresponding to RNA species U5, hY3, and hY5 were seen with a serum from a patient with generalized onchocerciasis. On the other hand, serum from a patient with localized onchocerciasis precipitated hY RNAs in propor-

Table I. Reactivity of Onchocerca volvulus Murine Monoclonal Antibodies with Purified Human Wil-2 Cell 46-kD Ro-SS-A Autoantigen (Calreticulin) and Ovalbumin

	ELISA optical density values	
Monoclonal antibody	Calreticulin	Ovalbumin
1. SNI G8	0.054	0.036
2. SNI C12	0.185	0.034
3. SNI G6	0.053	0.030
4. SNIII B6	0.069	0.032
5. SNII B5	0.058	0.028
6. SNII C6	0.329*	0.031
7. SNII A7	0.080	0.032
8. SNIII G5	0.059	0.043
9. SNIV A7	0.074	0.039
10. SNV E3	0.077	0.035
11. SNV B6	0.096	0.030
12. SNV C3	0.092	0.032
13. SNV G8	0.067	0.028
Culture medium control	0.064	0.041

* Greater than two standard deviations above the mean value produced by the culture medium control.

tions similar to that seen with the SCLE sera. Altogether, three onchocerciasis sera (two localized, one generalized) precipitated only hYRNA; two sera (one localized, one generalized) precipitated a heterogeneous mixture of hYRNA and other RNA species; and two sera (one localized, one generalized) did not precipitate hYRNA. In addition, an African control serum, like the Black U. S. control serum, did not precipitate hYRNA. Precedence for selective immunoprecipitation of certain hY RNAs by specific sera was established in a study that demonstrated the selective immunoprecipitation of hY5 by two SLE patient sera (27).

Northern filter hybridization. A 24-mer oligonucleotide probe complementary to hY3 RNA was radiolabeled and hybridized with nonradiolabeled RNA derived from immunoprecipitates of WiL-2 cell extracts, as shown in Fig. 3 *B*. The immunoprecipitate of a single localized onchocerciasis serum that precipitated labeled hYRNA also hybridized with the hY3 RNA probe. Two other immunoprecipitates produced by onchocerciasis sera did not hybridize with the hYRNA probe. Hybridization was not seen with immunoprecipitates using normal and anti-Sm antibody-containing sera (data not shown).

To exclude the possibility that the results of the hY RNA immunoprecipitation studies represented precipitation of heterologous RNA species of similar molecular weights, serial immunoprecipitation studies were performed in which radiolabeled WiL-2 cell lysates were preadsorbed with SCLE sera, as seen in Fig. 4, and the resulting supernatants were subject to immunoprecipitation by sera from an onchocerciasis patient previously shown to precipitate hY RNAs. The reverse experiment was also performed with the same sera. In this case, the precipitation with onchocerciasis sera preceded that with SCLE sera. The absence of significant immunoprecipitation after the initial preclearing step in both experiments confirms the identity of the RNA species associated with the antigens precipitated by sera of both sources.

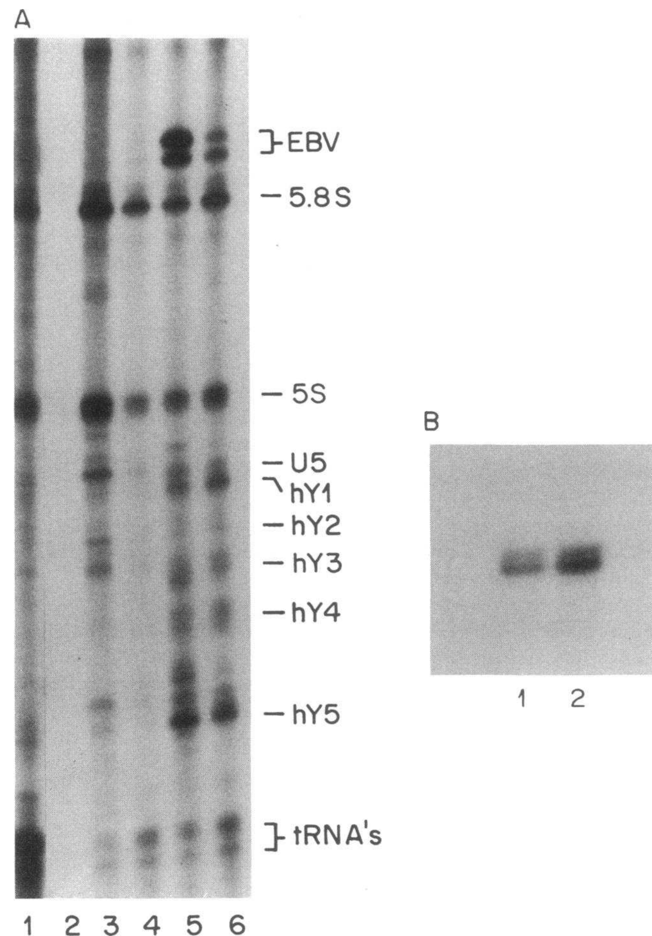

Figure 3. (*A*) Autoradiogram of electrophoretic gel of nucleic acids derived from immunoprecipitates of [^{32}P]orthophosphate-labeled WiL-2 cell extracts. Lane *1* represents total RNA before immunoprecipitation. The sera used were of the following sources: normal American Black (lane *2*), generalized onchocerciasis patient (lane *3*), localized onchocerciasis patients (lanes *4* and *5*), and SCLE (lane *6*). The labels at the right correspond to the RNA species immunoprecipitated. (*B*) Northern filter hybridization. A 24-mer oligonucleotide probe complementary to hY3 RNA was radiolabeled and hybridized with RNA derived from immunoprecipitates of WiL-2 cell extracts using sera from a patient with SCLE (lane *1*) and onchocerciasis (lane *2*).

Discussion

A number of observations suggest that calreticulin is an integral component of the human Ro/SS-A autoantigen system.

Virtually all of the Ouchterlony- or CIE-defined monospecific anti-Ro/SS-A sera from SCLE and SS patients that we have examined thus far have antibodies that react with purified native human WiL-2 cell calreticulin by CIE and ELISA (28).[2] Amino acid sequencing of multiple staphylococcal V-8 protease- and cyanogen bromide-derived cleavage fragments of purified WiL-2 cell calreticulin have confirmed that this puri-

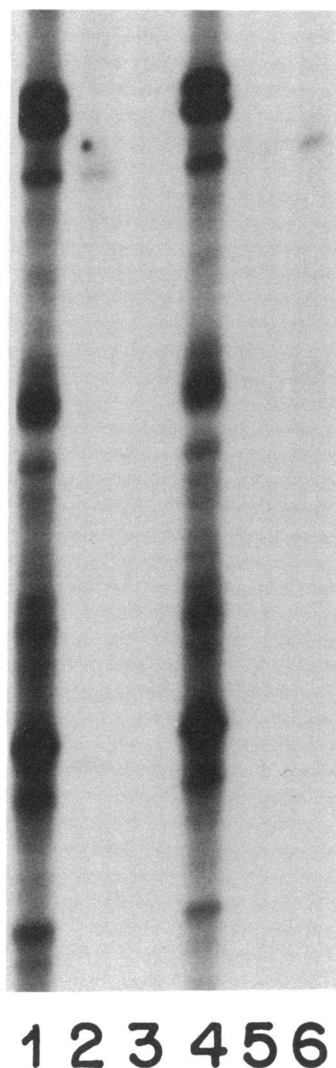

Figure 4. Autoradiogram of electrophoretic gel of serial immunoprecipitates of two radiolabeled WiL-2 cell lysates. The first extract was precleared twice with SCLE sera (lanes *1* and *2*), and the resulting supernatant was precipitated with the sera of a patient with onchocerciasis (lane *3*). A similar WiL-2 cell lysate was twice precleared with onchocerciasis sera (lanes *4* and *5*) and the remaining supernatant was immunoprecipitated with sera from a patient with SCLE (lane *6*).

fied antigen is not contaminated by other proteins, such as the 60-kD Ro/SS-A autoantigen (13, 26).

80% of Ro/SS-A precipitin-positive SCLE and SS patient sera tested react strongly by ELISA to two of three synthetic peptides that correspond to different portions of the human calreticulin sequence (28).

Purified native WiL-2 cell calreticulin also reacts with the Centers for Disease Control anti-Ro/SS-A reference serum (AF-CDC7) by CIE and Western blot analysis (29).[2]

An affinity-purified rabbit antiserum raised against a synthetic peptide that corresponds to amino acids 6-19 of the human calreticulin sequence immunoprecipitate all four hYRNAs (13). In addition, ultraviolet light cross-linking studies suggest that native human WiL-2 cell calreticulin is directly bound to a set of four RNA molecules similar in size to the hYRNAs (13).

A goat antiserum reactive with rabbit skeletal muscle calreticulin and a rabbit antiserum raised against a synthetic peptide corresponding to the 6-19 amino acid sequence of the human WiL-2 46-kD Ro/SS-A antigen (calreticulin) produce identical patterns of endoplasmic reticulum-like perinuclear fluorescence in formaldehyde-fixed and saponin-permeabilized cultured human epidermal keratinocytes (29–30).

The studies described in this report that were originally presented in abstract form in 1990 (31) demonstrate the existence of antibodies reactive with the human 46-kD Ro/SS-A autoantigen (calreticulin) in patients with onchocerciasis by immunoblotting, hY RNA immunoprecipitation, and northern blot analysis with an hY3 specific oligonucleotide probe. The amino acid sequence homology between the λRal-1–encoded protein and the 46 Ro/SS-A protein, particularly in an area of sequence triplication that occurs between amino acids 207 and 255 of the Ro/SS-A sequence, probably account for this antigenic cross-reactivity (13). The distribution of sequence similarity between these two molecules may explain the observed differences in their immunoreactivity. Specifically, some onchocerciasis sera bound to the 37-kD carboxy-terminal fragment by immunoblot after cleavage of the 46 Ro/SS-A protein by staphylococcal V8 protease, while sera from patients with SCLE and SS predominantly bound to the 23-kD fragment.

The common appearance of ANA and RF in patients with various viral, bacterial, and parasitic infections has been attributed to polyclonal activation of B cells with resultant cross-reacting autoantibody production (32). The absence of ANA and RF in the sera of patients with onchocerciasis is intriguing and is perhaps related to a focal production of antibodies cross-reactive with onchocercal and human antigens by a limited number of clonal lymphocyte populations.

The failure of onchocerciasis patient sera to form a line of precipitation with the purified 46-kD Ro/SS-A protein by either immunodiffusion of CIE was unexpected, given our previous successful experience in these assays using both our own monospecific Ro/SS-A reference sera as well as the monospecific Ro/SS-A reference serum from the CDC (AF-CDC7). Perhaps epitopes shared by *O. volvulus* and Ro/SS-A antigens are inaccessible to antibody binding on the native configuration of the Ro/SS-A protein used in these precipitin assays. Another possibility is the recognition by onchocerciasis sera of a single epitope on the Ro/SS-A protein that would preclude lattice formation and subsequent immune complex precipitation. Our immunoblot data, however, argue against this latter possibility.

The demonstration that an *O. volvulus* murine monoclonal antibody raised independently in another laboratory reacted with native human WiL-2 cell 46-kD Ro/SS-A autoantigen (calreticulin) further suggests that the λRal-1 *O. volvulus* antigen and human calreticulin share cross-reactive epitopes.

Both at a clinical level and a histologic level, the findings present in onchocerciasis are quite different from those seen in SCLE. The pathologic appearance of the latter may include pigment incontinence, mucin deposition, and hyperkeratosis, but in contrast to onchocerciasis, does not often have eosinophilic infiltrates or striking deep dermal edema. The liquefactive changes present in the epidermal basal cell layer of SCLE lesions are not seen in onchocerciasis skin disease. In addition, there is at this time no evidence that other clinical manifestations associated with anti-Ro/SS-A autoantibody production such as SS and neonatal lupus erythematosus (LE) occur in onchocerciasis patients. However, an interesting similarity between these two disorders lies in their HLA associations. Both diseases are associated with HLA-DRw52, and this association

is especially strong for the localized form of onchocerciasis (33). Similarly, Ia antigens have been found to be an important factor in infection susceptibility in a murine model for ascaris infection (34).

While the onchocerciasis patients studied in this report have no clinical evidence of autoimmune disease related to their antibodies that are crossreactive with the 46-kD Ro/SS-A (calreticulin) antigen, similar immune responses against parasite antigens may be responsible for the induction or exacerbation of autoimmunity in a subset of patients. In the U. S., related human nematode infections that could potentially lead to the development of a cross-reactive Ro/SS-A autoimmune response include *Enterobius vermicularis* (pinworm), *Dirofilaria immitis* (canine heartworm), *Necator americanus* (hookworm), *Ascaris lumbricoides,* and *Trichinella spiralis.* Another example of this potential pathologic mechanism is the finding of sequence homology between the $p30^{gag}$ antigen of some mammalian retroviruses and that of DNA topoisomerase I. Antibodies to the latter protein are characteristic of diffuse systemic sclerosis. It is speculated that an immunologic response to such a viral protein could be a factor in the development of this autoimmune disorder (35).

Other workers have recently confirmed that autoantibodies to an *E. coli*-expressed form of recombinant calreticulin can frequently be found by ELISA in unselected systemic LE (36). In addition, another laboratory has recently confirmed that onchocerciasis sera contain antibodies that react with *E. coli* recombinant human calreticulin (37). Neither group, however, could demonstrate significant reactivity of this form of calreticulin, as well as an in vitro translated form of the molecule, with human anti-Ro/SS-A patient sera. In earlier studies, our group also had difficulty showing reactivity of *E. coli*-expressed recombinant and in vitro translated forms of human calreticulin with anti-Ro/SS-A sera (personal observations). These results further emphasize that, as has recently been suggested for the 60-kD Ro/SS-A polypeptide (38), conformational epitopes appear to be the structures on calreticulin that are primarily targeted by the human autoimmune response. An alternative possibility is that some form of posttranslational modification that does not occur in a prokaryotic in vitro expression system is necessary for full display of the structures on calreticulin that are targeted by the human autoimmune response.

In summary, we have demonstrated the existence of antibodies reactive with the human 46-kD Ro/SS-A autoantigen (calreticulin) in patients with onchocerciasis and raise the possibility that infectious organisms such as *O. volvulus* may play a role in triggering or exacerbating at least one component of the human Ro/SS-A autoimmune response. This data also further supports our earlier data suggesting that the 46-kD Ro/SS-A protein (calreticulin) is directly associated with the hY RNAs. The association between calreticulin and the other polypeptide components of the Ro RNP remains to be determined.

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