Molecular Characterization of the Ro/SS-A Autoantigens

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Molecular techniques have recently revealed that there are several immunologically distinct Ro/SS-A antigens. Three genes encoding putative Ro/SS-A protein antigens with calculated masses of 46, 52, and 60 kD have been isolated. The encoded amino acid sequence of each is quite dissimilar. The 46-kD antigen is calreticulin (CR), a highly conserved calcium-binding protein that resides predominately in the endoplasmic reticulum where it may be involved in protein assembly. Although CR has recently been confirmed to be a new human rheumatic disease-associated autoantigen, its relationship to the other components of the Ro/SS-A ribonucleoprotein has become somewhat controversial owing predominately to the fact that recombinant forms of calreticulin have not displayed the same pattern of autoantibody reactivity possessed by the native form of this protein. The 52-kD antigen most likely resides in the nucleus and may be involved in the regulation of gene expression. The cellular location and function of the 60-kD antigen is uncertain but studies indicate that it is a RNA-binding protein. The 46- and 60-kD antigens share homology with foreign polypeptides, suggesting that an immune response initially directed against a foreign protein may give rise to the autoimmune response directed at cross-reacting self proteins. *J Invest Dermatol 100:73S–79S, 1993*

In 1969 Clark *et al* first demonstrated a novel antigen in human tissue extracts that by immunodiffusion analysis reacted with sera from patients with systemic lupus erythematosus and Sjögren's syndrome [1]. This antigen and its reactive autoantibodies were called Ro (auto)antigen and Ro (auto)antibodies, respectively. In 1975 Alspaugh and Tan similarly demonstrated the presence of three different autoantibodies in sera from patients with Sjögren's syndrome that they designated SS-A, SS-B, and SS-C [2]. Later it was demonstrated that SS-A autoantibodies were immunologically equivalent to the Ro autoantibodies [3], and thus we now commonly preface these autoantibodies and their respective antigens with the term Ro/SS-A. SS-B autoantibodies were also shown to be identical to La autoantibodies, a specificity that frequently accompanies Ro/SS-A autoantibodies [3].

CLINICAL SIGNIFICANCE OF THE Ro/SS-A ANTIGENS AND ANTIBODIES

The Ro/SS-A antigens are of clinical interest in that antibodies directed against them are found in the majority of patients with primary Sjögren's syndrome, subacute cutaneous lupus erythematosus (SCLE), neonatal lupus erythematosus (NLE), anti-nuclear antibody (ANA) negative lupus erythematosus (LE), and systemic LE-like disease secondary to homozygous C2 or C4 complement deficiency [4–9]. Substantial evidence indicates that they play a major role in the pathogenesis of disease [10,11]. The strongest evidence comes from observations in patients with NLE. Pregnant mothers with circulating Ro/SS-A autoantibodies can pass them across the placenta to their fetus. The fetus can develop congenital heart block, hepatic inflammation, and thrombocytopenia from tissue injury presumably caused by these antibodies [12,13]. Additionally, shortly after birth and perhaps triggered by ultraviolet (UV) light exposure, these infants can develop skin lesions clinically and histopathologically similar to those of SCLE [12]. In several months, as the maternally acquired antibodies are cleared from the infant's circulation, the skin lesions resolve [12].

Investigative work has provided additional evidence that Ro/SS-A antibodies may be pathogenic. Ro/SS-A antibodies administered intravenously to immunodeficient mice engrafted with human skin bind preferentially in and about the human basal keratinocytes [14]. This binding is augmented by UV light exposure. This pattern of immunoglobulin deposition is identical to that found in biopsies from NLE and SCLE skin lesions.

CHARACTERIZATION OF THE Ro/SS-A ANTIGENS

Since Clark *et al* first demonstrated the presence of Ro/SS-A autoantibodies by immunodiffusion studies in 1969[1], we have learned a great deal more about their target antigens.

In 1981 Lerner *et al* demonstrated that human Ro/SS-A autoimmune sera immunoprecipitated a novel class of small RNAs that they designated the human (h) cYtoplasmic (Y) RNAs or hY RNA [15]. In 1984 Wolin and Steitz showed that this hY RNA immuno-precipitation resulted from the binding of Ro/SS-A autoantibodies to a 60-kD protein to which the hY RNAs were apparently linked [16]. From 1984 until 1988 it was generally thought that Ro/SS-A autoantibodies were directed at a single 60-kD protein. However, in 1988 Ben-Chetrit *et al* demonstrated a novel 52-kD Ro/SS-A antigen by immunoblot analysis that was immunologically distinct from the 60-kD antigen [17]. In 1989 Rader *et al* demonstrated four immunologically distinct antigens that react with monospecific Ro/SS-A autoimmune sera [18]. Over the past four years genes encoding 60-, 46-, and 52-kD autoantigens have been isolated.

60-kD Ro/SS-A In 1988 Deutscher *et al* reported the cloning of a 60-kD Ro/SS-A antigen from a human placental complementary (cDNA) library [19]. In 1989 Ben-Chetrit *et al* [20] reported the cDNA sequence cloned from a human T-cell leukemia cDNA library that appeared to be a

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Abbreviations: ANA, anti-nuclear antibody; CDNA, complementary DNA; CMV, cytomegalovirus; CR, calreticulin; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; GRP, glucose-regulated protein; hY RNA, human cytoplasmic RNA; LE, lupus erythematosus NLE, neonatal lupus erythematosus; PDI, protein disulfide isomerase; RNP, ribonucleoprotein; SCLE, subacute cutaneous lupus erythematosus; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; UV, ultraviolet; VSV, vesicular stomatitis virus

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homologous gene or a differentially spliced version of the gene characterized by Deutscher *et al*. Deutscher's and Ben-Chetrit's sequences both encoded a 60-kD protein that was identical except for several amino acids at the carboxy-terminus. Both amino acid sequences contain a zinc finger motif and a sequence motif common to RNA binding proteins [19,20]. Zinc fingers are thought to serve as a site of nucleic acid binding or protein binding [21].

There is no extensive sequence homology between the 60-kD protein and other published protein sequences; however, six segments of this protein have limited homology to different portions of a vesicular stomatitis virus (VSV) nucleocapsid protein [22]. Five of these homologous regions have significant reactivity to human Ro/SS-A autoimmune sera by enzyme-linked immunosorbent assay (ELISA), which suggests that an immune response initially directed at the VSV protein or a similar viral protein might cross-react with the 60-kD Ro/SS-A protein by way of its cross-reacting epitopes. VSV is not known to be particularly pathogenic in humans, but the data suggest that this VSV protein or a similar viral protein might elicit the Ro/SS-A autoimmune response. This is an example of ''molecular mimicry'' where a microbial protein is thought to trigger an immune response that cross-reacts with a self-antigen [23].

46-kD Ro/SS-A Our group isolated a cDNA from an Epstein-Barr virus transformed human B-cell line that encodes a protein reactive with human Ro/SS-A autoimmune sera [24]. The encoded 46-kD protein migrates aberrantly at 60 kD by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The amino acid sequence of this 46-kD molecule reveals a hydrophobic leader sequence at its amino terminal typical of molecules transported into the endoplasmic reticulum (ER), and a KDEL carboxy terminal sequence that is classic for proteins that are retained in the ER [24,27]. This sequence is extremely homologous to murine and rabbit CR (94% and 92%, respectively) [28]. CR is a calcium-binding protein that resides in the endoplasmic and sarcoplasmic reticulum [29]. From this high degree of sequence homology and other data it has been concluded that the 46-kD molecule is human CR [28,30].

Recent evidence indicates that CR may be coordinately expressed with the glucose-regulated protein (GRP)78, GRP94, and protein disulfide isomerase (PDI) genes [31]. The protein products of all four of these genes are highly acidic, localize to the ER, and bind calcium [32]. GRP78 and GRP94 are highly homologous to heat-shock proteins [33,34]. Both of these proteins and PDI are thought to play a major role in protein assembly within the ER [33–35]. Thus, by implication, CR may also play a role in protein assembly.

It is also interesting to note that patients infected with *Onchocerca volvulus,* a filarial nematode that causes river blindness, sclerosing lymphadenitis, and dermatologic disease in humans residing in parts of Africa and Central America, have antibodies directed against the Ral-1 antigen, which is highly homologous to CR [28,36]. Although patients with this nematode are not at increased risk for developing Sjögren's syndrome or LE, this homology between CR and a filarial protein raises the possibility that a foreign Ro/SS-A protein equivalent might trigger the autoimmune Ro/SS-A antibody response seen in some patients with Sjögren's syndrome and several LE-related disorders. *Drosophila melanogaster* and the marine snail *Aplysia californka* have molecules similar to CR [28].

Work recently presented by other laboratories has now confirmed our initial suggestion that calreticulin is a rheumatic disease-associated autoantigen [37,38]. One group has found that an *Escherichia coli* recombinant form of human calreticulin reacted by ELISA with 40% of the serum samples from unselected SLE patients [37], whereas another group found 33% of their SLE sera to react with the same form of recombinant CR by Western blot [38]. Curiously, neither group found a significant correlation between anti-CR and anti-Ro/SS-A autoantibody levels in their SLE patient sera.

We have reason to believe that post-translational modification such as phosphorylation, perhaps through the augmentation of hY RNA binding, could also be relevant to the configuration of CR that is recognized by Ro/SS-A autoantibodies. Non-mammalian forms of recombinant CR are unlikely to possess the same pattern of RNA binding or phosphorylation that is displayed by the native configuration of CR present in mammalian cells. The concern that CR may not be a component of the Ro/SS-A RNP complex stems from the fact that *recombinant* forms of CR both in our laboratory (personal unpublished observation) and in others [37,38] do not display the same degree of reactivity with Ro/SS-A autoantibodies that has previously been demonstrated with *native* human CR purified from human Wil-2 cells. We now have evidence that the protein-RNA binding site present on a subpopulation of CR molecules that bind hY RNA contributes to the structure that is being recognized by human anti-Ro/SS-A autoantibodies [39].

Table I. Characteristics of Three Ro/SS-A cDNA-Encoded Products

Protein	Location	Function	Homology	Pathogenic Role
60 kD	Nuclear? Cytoplasmic?	RNA binding	VSV nucleocapsid protein	Molecular mimicry?
46 kD	Predominantly cytoplasmic ± nuclear	Ca^{++} binding Protein folding?	Calreticulin Ral-1	Translocation Parasite homology?
52 kD	Predominantly nuclear ± cytoplasmic	Gene regulation?	rfp, rpt-1	

52-kD Ro/SS-A Chan *et al* and Itoh *et al* independently isolated the same cDNA sequence from two different human T-cell cDNA libraries that encodes a 52-kD protein reactive with human Ro/SS-A autoimmune sera [40,41]. The amino acid sequence predicted by this cDNA contained leucine zipper and zinc finger binding motifs and also had significant homology with the human ret transforming protein and the murine T-cell regulatory protein rpt-1, which are thought to play a role in gene regulation [40,41]. These sequence motifs and homologies suggest that the 52-kD Ro/SS-A molecule may be involved in gene regulation and thus probably resides in the nucleus.

There are no significant sequence homologies between the 60-, 46-, or 52-kD antigens (Table I). Despite the knowledge gained from analyzing the encoded amino acid sequences of each Ro/SS-A cDNA, there still remain a number of unanswered questions that several investigators have started to address.

Are These Antigens Structurally Associated and What Are Their Relationships to the hY RNAs? Ro/SS-A autoimmune sera from patients with LE and Sjögren's syndrome immunoprecipitate four hY RNAs (hYl, 3, 4, and 5) from human cell extracts [15] (see Fig 1). Antisera specific for either a 52-kD or a 60-kD protein have been shown to immunoprecipitate the hY RNAs from cellular extracts. However, in one study whenever 52-kD specific antibodies (not reactive to the 60-kD antigen by immunoblot analysis) were used, the hY RNAs and 52-kD protein were precipitated along with a small amount of 60-kD protein [17]. Thus it is not certain whether 1) the 52-kD species binds the hY RNAs directly; 2) the 52-kD protein is indirectly associated with the hY RNA through a direct association with a 60-kD hY RNA binding protein; or 3) the antibodies that appeared specific for the 52-kD antigen by immunoblot may recognize a cross-reactive epitope on the native Ro/SS-A ribonucleoprotein (RNP) particle.

Deutscher *et al* have demonstrated that the 60-kD Ro/SS-A protein directly associates with hY RNAs in reconstitution studies [19]. There is some evidence that CR associates with the hY RNA as suggested by UV cross-linking studies [24], immunoprecipitation of hY RNA by affinity-purified CR antisera [24], and the fact that onchocerciasis patient sera with CR reactive antibodies can immunoprecipitate hY RNA [42]. Additionally, preliminary data indicate that rabbit antisera raised against two synthetic peptides corresponding to different portions of the CR

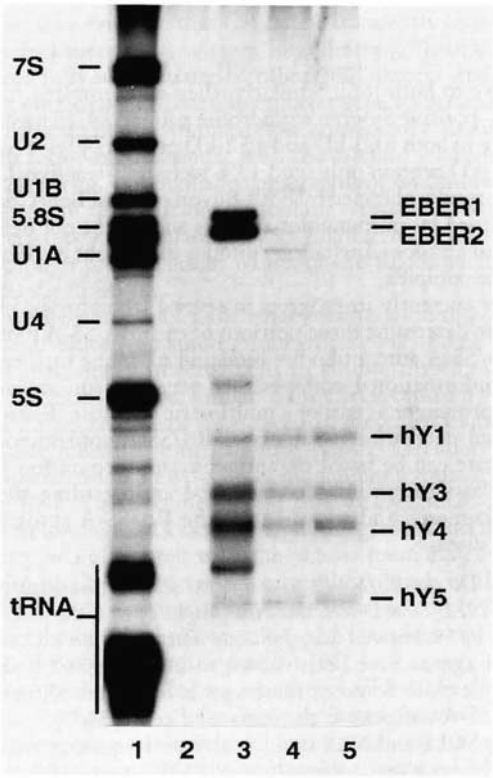

Figure 1. hY RNA immunoprecipitation. Wil-2 cells, an EBV transformed B-cell line, were radiolabeled with ^{32}P-orthophosphoric acid and sonicated. The resulting cell extract was incubated with sera and the immunoprecipitated material was harvested and subjected to gel electrophoresis according to previously established protocols [15]. *Lane 1* contains total radiolabeled RNA from the extract and serves to provide molecular weight standards as indicated to the left of this lane. *Lane 2* demonstrates that no RNA was immunoprecipitated with normal human serum. *Lane 3* is RNA immunoprecipitated with human autoimmune sera containing both Ro/SS-A and La/SS-B autoantibodies. *Lanes 4* and 5 are sera from two different patients with Ro/SS-A autoantibodies as denned by double immunodiffusion. All three sera with Ro/SS-A autoantibodies immunoprecipitated the four major types of hY RNA. The very faint unlabeled band in *lane 3* between hYl and hY3 is hY2, a breakdown product of hYl. The Epstein-Barr virus encoded EBER 1 and 2 immunoprecipitate with La/SS-B antibodies as previously described [56].

amino acid sequence can precipitate hYRNA [39]. However, the relationships that exist between CR and the other molecular constituents of the Ro/SS-A RNP particle continue to be debated [38,43,44].

Earlier data suggested that Ro/SS-A autoimmune sera precipitated a 60-kD protein along with the hY RNA and this immunopre-cipitation was dependent on the presence of protein [15]. More recently Boire and Craft have identified Ro/SS-A antibodies that specifically immunoprecipitate hY5 RNA and a 60-kD protein, but not the other types of hY RNA [45]. They concluded that some Ro/SS-A autoimmune sera contain auto-antibodies directed at a con-formational epitope that is expressed only on Ro/SS-A-hY5 RNA particles but absent on hY1, 2, 3, and 4 containing particles.

The 60-kD protein immunoprecipitated in the above studies most probably represents the authentic 60-kD protein as its amino acid sequence contains a sequence motif typically found in RNA-binding proteins, although it is clear that proteins without the classic RNA-binding consensus sequence can also bind RNA molecules [46,47]. The hY RNAs and Ro/SS-A proteins might exist in a RNP multimeric complex similar to the Ul RNPs. One could hypothesize that the zinc finger motif in the 60-kD Ro/SS-A molecule might serve as a site for this protein to

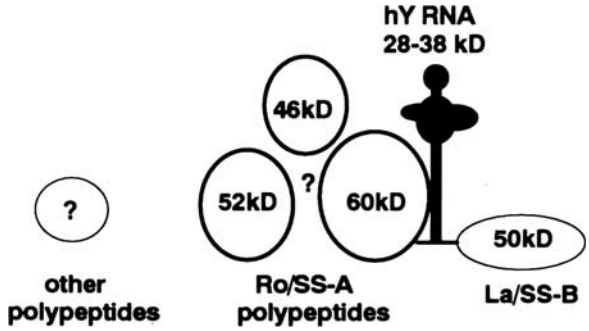

Figure 2. One model of the Ro/SS-A RNP particle. The proposed relationship between hY RNA and the Ro/SS-A and La/SS-B proteins is indicated. The hY RNA are RNA polymerase **III** transcripts and are thought to be at least transiently associated with the La/SS-B protein through binding of its uridine rich 3′ end. The 60-kD Ro/SS-A protein contains an RNA binding motif believed to be the site of hY RNA binding. It is uncertain if the 46- and/or 52-kD proteins reactive with Ro/SS-A autoimmune sera or other polypeptides are a part of this particle, although there is some evidence that the 46-kD protein is associated with hY RNA.

bind one or more of the other Ro/SS-A molecules, whereas the RNA binding motif would serve as a site of hY RNA binding.

Several different investigators have reported different masses for the Ro/SS-A RNP particle. Three groups have reported a mass of approximately 100–150 kD for the Ro/SS-A particle as determined by gel filtration [1,48,49]. Wolin and Steitz determined that the particle sediments in sucrose at approximately 7S [16]. This is equivalent to ~93 kD, which could be accounted for by the mass of one 60-kD protein and the average mass of one hY RNA molecule. Boire and Craft biochemically purified Ro/SS-A particles and found them to partition into three different groups by sucrose sedimentation gradients [50]. The particles in two of these groups have a mass of approximately 300–350 kD by gel filtration and the other has a mass of approximately 230 kD. These investigators concluded that Ro/SS-A RNP particles are heterogeneous and may exist as multimeric units consisting of hY RNA, a 60-kD protein, and perhaps other polypeptides including the La/SS-B protein [50] (see Fig 2).

Some investigators argue that if a particular protein reactive with Ro/SS-A autoantibodies is not part of the hY RNA-bearing RNP complex, then perhaps it should not be called a Ro/SS-A antigen. Although this is a matter of semantics, there is a clear need to develop a more precise designation for each of these antigens. However, it should be remembered that Ro/SS-A antigens were initially defined by immuno-diffusion assays, not by hY RNA association. It has not yet been determined which of the molecules reactive with Ro/SS-A autoimmune sera are responsible for the immunoprecipitate seen in immunodiffusion assays. Rader *et al,* however, observed that non-reducing SDS-PAGE of eluted precipitins from counter-immunoelectrophoresis showed the precipitins to be composed of a 60-kD protein [18].

What Is the Cellular Distribution of Each Ro/SS-A Molecule? Some investigators have reported predominantly intranuclear localization of the Ro/SS-A antigens by indirect immunofluorescence [51–54] whereas others have reported cytoplasmic [55–57] or both cyto-plasmic and nuclear localization [58,59]. Whether this discrepancy in subcellular localization is related to the method of cell fixation, cell substrate, or the specificity of the Ro/SS-A autoimmune sera used has not been fully investigated.

Koch *et al* have demonstrated that conventional cell fixation for ANA testing does not allow full visualization of proteins, such as CR, that reside in the ER [60]. They demonstrated that ER proteins can be better visualized by immunofluorescence techniques with detergent permeabilization of fixed cells or of unfixed cells that have been equilibrated in 9% sucrose [60].

Isolation of antibodies specific for each Ro/SS-A antigenic polypeptide has allowed more precise cellular localization of the Ro/SS-A polypeptides and these results have helped explain the discrepancies encountered previously with immunofluorescence staining using whole Ro/SS-A patient sera.

Ben-Chetrit *et al* demonstrated strong punctate nuclear and slight cytoplasmic staining of cells with Ro/SS-A autoimmune serum immunoaffinity purified from a 52-kD protein and also with monospecific anti- 52-kD serum (as determined by immunoblot analysis) [17]. They reported an indistinguishable pattern with Ro/SS-A autoimmune serum immunoaffinity purified from a 60-kD protein [17]. There is some evidence that there may be Ro/SS-A antibodies that react with an epitope on the native 60-kD RNP antigen that cross-react with an epitope on the 52-kD protein [61]. Thus one might speculate that the 60-kD protein resides primarily in the cytoplasm and that nuclear staining results from antibodies that recognize a cross-reactive epitope on the 52-kD protein. Preliminary ELISA data have revealed that all Ro/SS-A autoimmune sera with high-titer antibodies directed against a recombinant 52-kD Ro/SS-A protein have given a speckled nuclear ANA pattern of fluorescence (personal unpublished observation). The 52-kD Ro/SS-A amino acid sequence reveals motifs and homologies that indicate that it may be involved in gene regulation and thus would likely reside in the nucleus [40,41]. Antiserum specific for CR has revealed predominantly perinuclear cytoplasmic staining with lesser amounts of nuclear staining [62,63].

Kato *et al* have reported that the majority of the hY5 RNA is found in the cytoplasmic fraction of HeLa cells [64]. More recently Boire and Craft have demonstrated that more than 90% of the Ro/SS-A particles were recovered from the cytoplasmic fraction of HeLa cells [50]. One should recall that Ro/SS-A antigen was originally described as a saline-soluble, ''cytoplasmic'' autoantigen [1].

Is There Any Correlation Between the Type of Ro/SS-A Autoantibodies Patients Produce and Their Diagnosis, Disease Course, and/or Response to Therapy? Preliminary data suggest that patients with Ro/SS-A autoantibodies might be clinically categorized according to the Ro/SS-A proteins or epitopes their sera recognize. The specificity of the maternally acquired Ro/SS-A autoantibodies may determine which infants develop NLE. Buyon *et al* have shown that the predominant antibody response in the NLE patient group with acquired heart block was directed against the 52-kD Ro/SS-A protein, although some patients also had antibodies directed against a 60-kD protein [65]. Ben-Chetrit *et al* have demonstrated that of 51 SLE patients who were Ro/SS-A positive by immunodiffusion, 47% had antibodies that recognized both a 60-kD and a 52-kD protein, 18% had antibodies that recognized a 60-kD protein only, and 35% were nonreactive to both [66]. Similarly, they demonstrated that of 47 Ro/SS-A -positive Sjögren's syndrome patients, 47% had antibodies reactive to both a 60-kD and a 52-kD protein, 40% reacted only with a 52-kD protein only, and 13% were non-reactive. Unfortunately the results obtained by both Buyon *et al* and Ben-Chetrit *et al* were obtained by immunoblot analysis and might not detect conformational epitopes, including epitopes that might be present on a multimeric complex.

Work is currently in progress in several laboratories, including our own, to determine those portions of each Ro/SS-A molecule to which Ro/SS-A autoantibodies bind and to define further the nature of conformational epitopes that may exist on an individual Ro/SS-A protein or as part of a multimeric structure. Epitope mapping should determine if subsets of Ro/SS-A autoantibody-associated disease can be based on antigenic site-recognition patterns. Such subclassification may have clinical use regarding the pathogenesis, diagnosis, and treatment of the Ro/SS-A autoantibody-associated diseases.

What Role Do the Ro/SS-A Autoantigens Play in the Pathogenesis of SCLE and NLE Skin Disease and How Might Their Cellular Expression Be Affected by Factors that May Influence These Two Skin Diseases? A number of agents have been shown to influence SCLE and NLE disease expression. Some of these agents have been shown to displace Ro/SS-A antigens to the surface of cells (Fig 3).

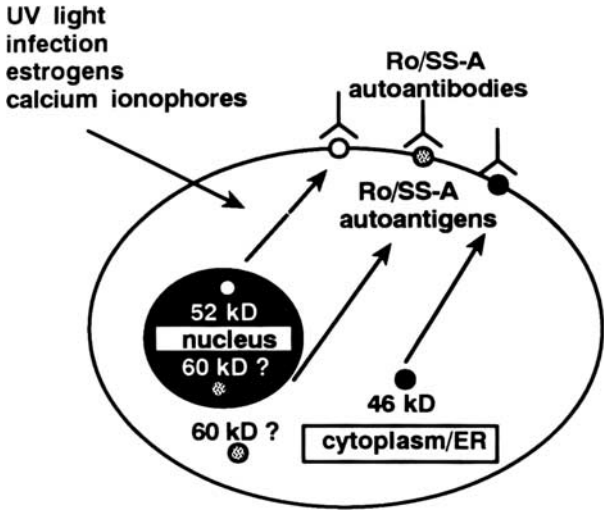

Figure 3. Ro/SS-A translocation. Agents that may translocate Ro/SS-A antigens to the cell surface where they are available to bind circulating Ro/SS-A autoantibodies are listed. The likely predominant location of the 46- and 52-kD antigens are indicated. The location of the 60-kD antigen is more uncertain.

UV light SCLE and NLE skin diseases are frequently exacerbated by UV light exposure, primarily of the UVB type (290–320 nM) [67,68]. Investigators have demonstrated that UVB light can displace Ro/SS-A antigen from within keratinocytes to the cell surface [69,70]. Similar results have recently been reported for the La/SS-B antigen [71]. Such displacement would allow the autoantigen to have access to the Ro/SS-A autoantibody binding that could result in tissue injury through complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity. At this time it is uncertain which of the various molecular components of the Ro/SS-A autoantigen complex are affected by UV light exposure. Recent preliminary studies have indicated that physiologically relevant doses of UVB induce both CR gene transcription [72] (personal observation) and translation [73]. However, similar doses of UVB did not increase cellular levels of the 60-kD Ro/SS-A protein [73]. No evidence has yet been presented regarding the displacement of the various Ro/SS-A proteins to the cell surface by UV light.

Estrogens The hormonal milieu may also play an important role in Ro/SS-A-associated disease, as approximately 75% of SCLE patients and infants with NLE skin disease are female. Estrogen treatment of cultured keratinocytes results in increased expression of Ro/SS-A antigens on the cell surface, but it is uncertain which of the Ro/SS-A molecules are so displaced [74].

Heat Some SCLE patients have noted that heat can exacerbate their skin disease [67]. The similarities that CR shares with the heat shock-like proteins GRP78 and GRP94 is of interest in this regard. There is recent evidence that heat shock – like proteins can be translocated to the cell surface, where they may participate in antigen presentation or may be a target for gamma/delta T-cell-directed cytotoxicity [75,76].

State of keratinocyte differentiation and proliferation Studies have shown that human keratinocytes grown in low-calcium-containing culture media have greater amounts of cytoplasmic Ro/SS-A antigen as detected by immunofluorescence staining with Ro/SS-A autoimmune sera [59]. It is uncertain which of the Ro/SS-A antigens are expressed in greater amounts, however. At lower calcium concentrations, cultured keratinocytes are in a less-differentiated, more rapidly proliferating state, more like the basal layer keratinocytes [77]. This might explain why the more rapidly proliferating basal keratinocytes appear to be preferentially targeted in SCLE and NLE [11,78]. Preliminary evidence indicates that CR

is expressed at higher levels in rapidly proliferating cells (personal unpublished observation).

There is some evidence that CR can be transiently secreted from cells after treatment with calcium ionophores [79], although this has not been fully substantiated by others [80]. Recent studies in our laboratory employing calreticulin reporter gene constructs have indicated that calreticulin transcription is markedly augmented in A431 cells following treatment with the calcium ionophore ionomycin [72] (personal unpublished observation). Additionally, it has been suggested that calcium ionophore treatment of cultured human keratinocytes displaces Ro/SS-A antigen to the cell membrane, although it is uncertain which of the Ro/SS-A antigens is (are) so displaced [81].

Infection There is considerable evidence that viral infections can precipitate or exacerbate autoimmune disease [82]. Some work has suggested that virally infected cells in culture displace Ro/SS-A antigens to the cell surface, similar to the effects caused by UV light [83]. Jianhui and Newkirk have recently presented evidence that suggests that human cytomegalovirus (CMV) infection increases the expression of CR in MRC-5, a human embryonic lung fibro-blast line [84]. These studies found that total cellular CR levels increased following CMV infection, but the greatest increase in CR antigenicity was associated with the plasma membrane. CMV infection also resulted in increased intracellular levels of the 60-kD Ro/SS-A antigen, but similar increases were not observed in the plasma membrane. Viral infection has also been shown to displace the La/SS-B antigen from the nucleus [85].

Polymorphism It is not known whether Ro/SS-A proteins are polymorphic. Preliminary studies have indicated, however, that the CR encoding gene is not highly polymorphic in normal individuals by restriction fragment length polymorphism analysis [24]. There is evidence of at least two forms of the 60-kD Ro/SS-A encoding gene [18–20]. Kutsch *et al* have recently reported variability in Ro/SS-A displacement to keratinocyte membranes after treatment with calcium ionophore or tumor necrosis factor alpha, which they suggest might arise from genetic differences in the keratinocyte donors [81]. It is possible that certain forms of a Ro/SS-A molecule may be more immunogenic, expressed at higher levels, or more easily displaced to the cell surface where it could participate in immune-mediated injury.

SUMMARY

With molecular techniques, we have recently learned that there are several immunologically distinct Ro/SS-A antigens. Three putative Ro/SS-A genes have been isolated and the encoded proteins from these three genes have been found to be quite dissimilar. CR, the 46-kD protein, appears to reside predominately in the endoplasmic reticulum/cytoplasm, whereas the 52-kD protein most likely resides in the nucleus. The structure of the 60-kD protein and at least one immunofluorescence study suggest that it may also reside predominantly in the nucleus, although if it is the authentic hY RNA binding protein, it should also reside in the cytoplasm. The Ro/SS-A RNP particles are heterogeneous multimeric complexes that may include more than one Ro/SS-A molecule, hY RNA, La/SS-B, and perhaps other currently undefined molecules.

Two of the putative Ro/SS-A proteins share significant homology with foreign proteins, suggesting that an immune response initially directed against a foreign protein, such as a viral protein, may give rise to the autoimmune response directed at cross-reacting self Ro/SS-A protein. This, plus the possibility that viral infection can alter the normal cellular distribution of Ro/SS-A antigens, presents the intriguing paradigm illustrated in Fig 4 where virus infection might have a dual impact on the Ro/SS-A autoimmune response.

Work is in progress to determine if there is an association between which Ro/SS-A molecules and/or epitopes are targeted by patient autoimmune sera and patient diagnosis, clinical course, and/ or response to therapy. We are hopeful that efforts to characterize the structures and functions of the Ro/SS-A antigens will further elucidate the pathogenesis of the Ro/SS-A autoantibody-associated diseases and aid in the development of better diagnostic and therapeutic modalities for those afflicted.

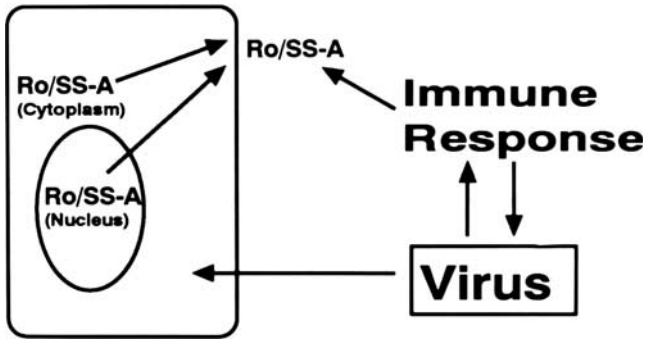

Figure 4. Model for a dual effect of virus infection on the Ro/SS-A autoimmune response. Infection with viruses that express molecules structurally associated with Ro/SS-A molecular subunits could result in translocation of Ro/SS-A antigens to the surface of cells. The immune response generated by the virus could then recognize the translocated Ro/SS-A antigens at the cell surface and mediate cellular damage through mechanisms such as complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity.

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