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Putative sequences on Ro60 three-dimensional structure accessible for 4-hydroxy-2-nonenal (HNE) modification compared to *in vitro* HNE modification of Ro60 sequences

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Abstract

We have previously reported accelerated acquisition of new autoreactivity upon immunization with 4-hydroxy-2-nonenal (HNE)-modified Ro60, as well as differential induction of lupus or Sjögren's syndrome by immunization with Ro60 containing varying amounts of HNE. Since the number of HNE molecules on Ro60 appears to be important, we hypothesized that specific sequences on Ro60 are targets for HNE-modification. Using surface plasmon resonance (SPR) we have also shown intramolecular protein-protein interaction between Ro60 and Ro multiple antigenic peptides (MAPs). We also hypothesized that intramolecular protein-protein interaction would be abolished by HNE- modification. To test this hypotheses we investigated (a) the epitopes of Ro60, using 19 Ro MAPs in an *in vitro* assay (involving HNE-modification of MAPs following immobilization on ELISA plates) to identify targets of HNE modification on Ro60 and (b) the protein-protein interaction between unmodified Ro60 MAPs, immobilized on the sensor surface of BIAcore, and unmodified Ro60 or HNE-modified Ro60 using SPR. New data obtained with SPR strengthens our earlier observation that immunization with HNE-Ro60 induces a stronger response. Unmodified Ro60 bound to several Ro60 MAPs through protein-protein interaction analyzed using SPR. This interaction was totally abrogated using HNE-modified Ro60 suggesting that sequences on Ro had become modified with HNE. When 19 Ro60 MAPs were modified *in vitro* with HNE, it was found that 10/19 MAPs significantly bound HNE covalently ($p < 0.001$ compared to MAPs binding HNE poorly). The amino acid sequences 126–137, 166–272 and 401–495 on Ro60 were strongly HNE modified. Using computational model system based on the recently published crystal structure for Ro60 enabled us to identify regions on the Ro60 molecule represented by the HNE-modified Ro MAPs, which are part of the exposed tertiary structure of the Ro60 protein.

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Keywords

4-hydroxy-2-nonenal; systemic lupus erythematosus; Sjogren's syndrome; Ro60; multiple antigenic peptides; surface Plasmon resonance; free radicals; lupus; autoimmunity; epitopes; autoantibodies; antigens; hydroxynonenal

1. Introduction

Systemic lupus erythematosus (SLE) is a clinically diverse chronic inflammatory disease of unknown aetiology (Kurien and Scofield, 2006). Environmental, hormonal and genetic factors are thought to play an important role in this disorder that affects women predominantly with a 9:1 ratio. Patients are unified in their disparateness of symptoms by the presence of autoantibodies to self-antigens. Antibodies to Ro60 (or SS-A) ribonucleoprotein (RNP) particle are found in the sera of up to 50% of SLE patients. Anti-Ro antibodies are highly prevalent in patients with Sjögrens syndrome, subcutaneous lupus erythematosus and mothers of infants with congenital lupus. On the other hand anti-La autoantibody is rarely found without anti-Ro (Scofield et al, 1999; Harley and Scofield, 1991; Harley et al, 1992).

The protein target of the anti-Ro60 antibodies in the RNP complex is Ro60, which interacts non-covalently with one of four short, uridine-rich human cytoplasmic RNA's (hYRNA's). In addition, the autoantigen La (or SS-B) is a stable constituent of the Ro-ribonucleoprotein. Though the particle has been shown to play an important role in the quality control discard pathway of 5S RNA, the complete role of the Ro complex has not yet been elucidated (Wolin and Reinisch, 2006).

Reactive oxygen species-mediated lipid oxidative damage is observed in SLE (Jiang and Chen, 1992; Serban et al, 1996; Michel et al, 1997; Turi et al, 1997; Grune et al, 1997; Kurien and Scofield, 2003; Vipartene et al, 2006; Kurien et al, 2006) and this topic has been reviewed recently (Kurien and Scofield, 2006; Kurien et al, 2008). Specifically, there is significantly elevated conjugated dienes and increased formation of malondialdehyde (Kurien and Scofield, 2003; Mansour et al, 2008), both indices of oxidative damage, in the sera of SLE patients compared to control. The damage is thought to be caused in part by the decrease in activity of the antioxidant enzymes, superoxide dismutase (Jiang and Chen, 1992; Kurien and Scofield, 2003; Vipartene et al, 2006; Zhang et al, 2008) catalase (Zhang et al, 2010) or glutathione peroxidase (Vipartene et al, 2006; Zhang et al, 2010;). The decreased activity of superoxide dismutase and catalase is possibly caused by the presence of significantly elevated antibodies against superoxide dismutase (Kurien and Scofield, 2003; Mansour et al, 2008) and catalase (Mansour et al, 2008) in SLE patients compared to normals.

Oxidative damage involves the interaction of free radicals with polyunsaturated fatty acids. This induces the formation of highly reactive electrophilic aldehydes, such as 4-hydroxy-2-nonenal (HNE), malondialdehyde, and acrolein. 4-hydroxy-2-nonenal (HNE) and malondialdehyde comprise the most abundant aldehydes, with acrolein being the most reactive. Proteins are especially vulnerable to covalent modification by HNE (Kurien et al,

2008; Perluigi et al, 2011). Each HNE molecule has two regions of reactivity, the alkene bond and the free aldehyde moiety. The alkene bond reacts with the three nucleophilic amino acids cysteine, histidine or lysine through Michael-type addition (Di Domenico et al, 2010). This reaction product is thought to be a cyclic hemiacetal, which equilibrates with an open-chain form of the protein adduct. The free electrophilic aldehyde, in the open-chain form of the alkenal adduct, can react with a second lysine, histidine or cysteine. Thus, HNE has the potential to act as heterobifunctional crosslinking reagent (Kurien et al, 2008; Kurien et al, 2011). Once HNE is bound to proteins it alters protein structure, protein function and activity. HNE has been shown to accumulate at concentrations of 10 μ M to 5 mM in response to oxidative stress (Perluigi et al, 2011).

Our laboratory observed increased free radical mediated oxidative damage in SLE (Kurien and Scofield, 2003) and also found proteins modified by HNE in human SLE. Therefore, we hypothesized that oxidative by-products, like HNE, would cross link with Ro60 and help initiate autoimmunity. To test this hypothesis, we immunized rabbits with either the HNE-modified Ro60 or the unmodified Ro60. We found that immunization of rabbits with HNE-modified Ro60 accelerated autoimmunity compared to those immunized with unmodified Ro60 (Scofield et al, 2005). Furthermore, we hypothesized that the level of HNE-bound to Ro60 would be important for the development of autoimmunity. We modified Ro60 with 0.4 mM (low), 2 mM (medium) or 10 mM (high) HNE and immunized mice with these antigens as well as unmodified Ro60 and saline as controls. We found that mice immunized with medium HNE-Ro60 developed a lupus like phenotype, while the mice immunized with the high HNE-Ro60 developed a Sjögren's syndrome like phenotype (Kurien et al, 2011).

Since, there was a differential disease induction with differential modification of Ro60, we hypothesized that the number of HNE molecules bound to Ro60 might be different. With that in mind we set out to analyze the number of HNE-molecules bound to low, medium or high HNE-modified Ro60 using matrix-assisted laser desorption-ionization time of flight mass spectrometry (Kurien et al, 2001). Unfortunately, we were unable to ionize the protein, and hence could not observe the difference in molecular weight based on number of HNE molecules bound to the low, medium or high HNE-modified Ro60, compared to the unmodified Ro60. Therefore, we hypothesized that Ro60 multiple antigenic peptides (MAPs) (Table 1) (Kurien et al, 1998), synthesized based on sequences on Ro60 that served as initial targets of lupus autoimmunity (Scofield et al, 1997; Huang, S-C et al, 1997), could serve as ideal targets for *in vitro* HNE-modification to study the normal degree of HNE-modification in the Ro60 molecule.

With that end in sight, HNE-modification of Ro60 MAPs was carried out *in vitro* in order to identify the sequences in the Ro60 protein that are involved in this modification. Since the crystal structure for Ro60 has been published recently (Stein et al, 2005), it enabled us to identify regions that are on the surface by use of computational models of the antigen. We hypothesized that these regions would be easily accessible for interaction with biomolecules *in vivo* and that these would be targets for HNE-modification. Interestingly most of the HNE-modified MAPs were located in regions that were easily accessible while those that were poorly modified were mainly in areas of the molecule that were mostly inaccessible.

Since we have observed specific protein-protein interaction between Ro60 and Ro60 MAPs we also hypothesized that this interaction will be abolished by the HNE-modification of the Ro60 autoantigen.

2. Materials And Methods

2.1. Instrumentation and reagents

BIAcore instrument, BIAevaluation software, carboxy methylated (CM) dextran-5 sensor chips (research grade), P20 surfactant and amine coupling kit were purchased from Pharmacia Biosensor AB, Uppasala, Sweden (The BIAcore instrument, and BIAevaluation software were available to us as part of the Core facility at OMRF) Serocluster “U” vinyl ELISA plates were from Costar, Cambridge, MA. Alkaline phosphatase conjugated affinity purified goat anti-human secondary antibody was from Jackson ImmunoResearch Laboratories, Bar Harbor, ME, USA. 4-hydroxy-2-nonenal (HNE) was purchased from Cayman Scientific, Ann Arbor, MI. Pre-stained protein molecular weight standards (high and low range) were from Bio-Rad, Richmond, CA, USA. Anti-HNE antibodies, purchased from Biotrend, were used as a control. Phosphate buffered saline tablets were from Sigma Chemical Co, MO. All other chemicals used were reagent grade.

2.2. Ro60 autoantigen

Purified bovine Ro60 (Dickey et al, 1993; Yamagata et al, 1984) was purchased from Immunovision, Springdale, AK).

2.3. Multiple antigenic peptides (MAPs)

Nineteen Ro60 multiple antigenic peptides (MAPs) were constructed from the sequence of Ro60 (Deutscher *et al*, 1988; Scofield *et al*, 2004). MAPs contain multiple copies of the same peptide sequence (eight copies) coupled covalently to a hepta lysine backbone (Kurien *et al*, 1998).

2.4. Matrix assisted laser desorption-ionization time of flight mass spectrometry (MALDI TOF MS)

Ro60 or HNE-Ro60 (1 or 10 µg/µl) was mixed with 0.1% TFA (1:1) and then diluted 1:1 with sinapinic acid (20 mg/ml) and spotted on a stainless steel grid and subjected to MALDI TOF MS (Kurien et al, 2004).

2.5. Surface plasmon resonance

BIAcore involves a sensor that is in contact with a microfluidic cartridge for surface plasmon resonance (SPR) detection. Ligand is coupled to a carboxy-methylated dextran matrix attached to the sensor surface. The other surface of the sensor is coated with a thin gold film and faces the optical system. The SPR detector responds to changes in the refractive index in the vicinity of sensor surface as the immobilized species interacts with its ligand in fluid phase. The sensor surface was prepared as follows. Phosphate buffered saline/P20 (Tween, supplied by Pharmacia Biotech) pH 7.4, was used as the eluent (running buffer) at a flow rate of 10 µl/minute during immobilization and all other qualitative

analyses. P20 was used at a concentration of 0.005% in the running buffer to reduce non-specific binding of protein to the sensor chip. The sensor chip was first equilibrated with PBS and then activated with 1:1 mixture of 0.1 M *N*-ethyl-*N*-(dimethylamino-propyl) carbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS). Then a specific MAP at a concentration of 100 µg/ml in 10 mM sodium acetate, pH 5.0, was injected. Remaining *N*-hydroxysuccinimide esters was deactivated with a pulse of 1 M ethanolamine, pH 8.5. For qualitative measurements about 3000–4000 response units (RU) of the MAPs were immobilized in different flow cells. Bovine Ro60 was diluted in the running buffer and analyzed over the MAP. The ligand surface was regenerated with 10 to 20 µl of 3M sodium thiocyanide. Typically, a single ligand surface was used for several analyses.

2.6. Preparation of Ro60 antigen for immunization

Modification of purified Ro60 in solution with 4-hydroxy-2-nonenal (HNE) (10 mM) was carried out as described previously (Scofield et al, 2005).

2.7. Rabbit immunization

Immunization of rabbits with HNE-modified and unmodified Ro60 was carried out as reported earlier (Scofield et al, 2005). Essentially two New Zealand White rabbits were immunized with HNE-modified Ro60 while four rabbits were immunized with unmodified Ro60. On day 1, 500 µg of either Ro60 or HNE-modified Ro60 was emulsified in 0.5 ml of complete Freund's adjuvant and injected intraperitoneally and subcutaneously. Subsequent boosts, with 500 µg antigen in incomplete Freund's adjuvant, were administered on day 26, 53, 99 with a final intravenous boost on day 152. The animals were bled weekly and sera obtained were stored at –20° C. All animals had pre-immunization sera collected.

2.8. In vitro modification of solid phase proteins by HNE

The modification of Ro60 MAPs following immobilization on ELISA plate was carried out as described earlier (Kurien and Scofield, 2005). Briefly, the Ro MAPs were coated at room temperature for two hours on ELISA plates in carbonate coating buffer. The plates were washed twice with phosphate buffered saline (PBS) pH 7.4 and 50 µl of HNE (200 µM) in PBS was added per well. Since HNE was supplied dissolved in ethanol, PBS with appropriate amount of ethanol added was used as control. This PBS solution (50 µl/well) was added for the protein fraction that served as unmodified controls. Following incubation for 4 hours at room temperature in a humid chamber, the plates were washed with PBS containing 0.05% Tween (PBST). The plates were blocked for two hours at room temperature with 3% milk/PBS and incubated with sample overnight at 4°C. The plates were then washed with PBST and incubated with appropriate secondary antibody for two hours at room temperature. Following washing, color was developed with p-nitrophenol phosphate substrate.

2.9. Computational analysis of Ro60 macromolecular structure to determine sequences accessible for HNE modification

The crystal structure of the Ro60 molecule [PDB ID: 1YVP] was analyzed in worm representation made by the MOLMOL molecular graphics program (Koradi et al, 1996).

3. Results

Owing to the importance of oxidative damage observed in SLE (Kurien and Scofield, 2003), and the role of by-products of oxidative damage such as HNE, we immunized four rabbits with unmodified Ro60 and two rabbits with HNE-modified Ro60. We hypothesized that HNE-modified Ro60 immunization would break tolerance much more rapidly than immunization with unmodified Ro60. The data regarding this experiment has been reported elsewhere (Scofield et al, 2005). Briefly, we observed rapid abrogation of autoimmunity in the HNE-Ro60 immunized animals compared to the unmodified Ro60 group. Anti-Ro60 response began by bleed 1 and bleed 2 respectively in the two HNE-Ro60 immunized animals, whereas it began only by bleed 7 and bleed 5 respectively for rabbits 1 and 2 immunized with unmodified Ro60. The anti-Ro60 response began by bleed 1 and 4, respectively in rabbits 3 and 4 immunized with unmodified Ro60. The level of anti-HNE-Ro60 response in the rabbits immunized with HNE-Ro60 was very significantly higher compared to the anti-Ro60 response in the animals immunized with unmodified Ro60 (Scofield et al, 2005).

In subsequent experiments in mice, we observed accelerated autoimmunity in mice immunized with Ro60 modified with either 0.4 (low), 2 (medium) or 10 (high) mM HNE, compared to mice immunized with unmodified Ro60. However, mice immunized with medium HNE-Ro60 developed antibodies to double stranded DNA and did not show diminished salivary flow characteristic of SS, thus displaying an SLE phenotype. However, the high HNE-Ro60 immunized mice did not develop anti-dsDNA antibodies, but had significantly reduced salivary flow and salivary gland lymphocytic infiltrates (Kurien et al, 2011). The differential effects observed based on the degree of HNE-modification prompted us to study the regions in the Ro60 molecule that rendered itself to modification with HNE.

In an effort to identify the targets of oxidative modification on the Ro60 autoantigen, we carried out an *in vitro* HNE-modification of 19 Ro MAPs (Table 1), which represent the B cell epitopes, to determine the sequences that are susceptible for modification with HNE. These MAPs have been used in several studies (Scofield et al, 1997, 1999, 2004; Scofield 2005; Kurien et al, 1998; Kurien and Scofield, 2005) and thus behave like a low molecular weight protein substrate for ELISA, immunoblotting or SPR analysis.

Figures 1–3 show the SPR analysis of rabbit anti-Ro and anti-HNE Ro interacting with the various Ro MAPs. It can be seen that, in general, sera from animals immunized with HNE-Ro bound the Ro-MAPs with higher affinity as seen (Scofield *et al*, 2005).

We had previously observed protein-protein interaction between Ro MAPs and Ro60 (Scofield *et al*, 1999). Two of the strongest of these were Ro60 with Ro-MAP-331 and Ro60 with MAP-482. Therefore, we were interested in finding out whether protein-protein interaction between Ro60 and Ro MAPs would be retained upon modification of the Ro protein with HNE. When Ro60 was analyzed over Ro-MAP-331 (MAP #16) we found that Ro60 bound to the immobilized MAP with a RU of over 200 (Figure 4B). However, this binding was abolished when HNE-modified Ro60 was passed over the same MAP (Figure 4A). A similar result was obtained for the interaction of the unmodified and HNE-modified

Ro60 protein with the Ro-MAP-482 (MAP# 21) coupled to the sensor surface (Figures 4D and 4C).

Next, we determined the sequences on the Ro60 protein that are susceptible to modification with HNE. For this purpose we used 19 Ro MAPs, and an unrelated MAP (PPPGMRPP) as control in an *in vitro* assay. Ro60 MAPs 4, 7, 9, 10, 11, 12, 13, 19, 20 and 21 (Table 1) bound HNE very significantly ($p < 0.001$ compared to Ro60 MAPs 2, 3, 6, 8, 15, 16, 17, 18 and the MAP PPPGMRPP (Table 1) from Sm autoantigen, which bound HNE very poorly. We analyzed these sequences by use of Ro60 computational model to determine sequences on the protein that are accessible for interaction with biomolecules *in vivo*.

Since cysteines, lysines and histidines are the targets of oxidative modification by HNE, we analyzed all the cysteines, histidines and lysines in the 19 Ro60 MAPs mentioned in Table 1. There are a total of 47 lysines, 13 histidines and 13 cysteines in the Ro autoantigen. By using computational models based on the crystal structure of the Ro autoantigen we showed that most of these amino acid residues were accessible when taken individually. Only histidines at position 179 (MAP#7), 257 (MAP#13), 320 (MAP#15) on the Ro60 autoantigen were not accessible while histidine at position 262 (MAP#13) was partially accessible. Similarly lysines at position 88 (MAP#2), 170 (MAP#7), 239 (MAP#12) and 495 (MAP#21) were only partially accessible.

MAP #7 has a histidine that is not accessible and a lysine that is partially accessible. However, it also contains two additional lysines that are accessible, explaining the reason for the observed significantly elevated HNE binding to this part of the MAP (anti-HNE binding with an OD of 1.84 ± 0.13 to HNE-modified MAP#7 compared to anti-HNE binding with an OD of 0.089 ± 0.004 to MAP#7 without HNE added) binding to Ro). Similarly MAP#13 has one histidine that is inaccessible and a lysine that is partially accessible. However, it also contains two additional lysines that are accessible, explaining the reason for the binding of HNE to the MAP (OD of 2.44 ± 0.2 vs 0.15 ± 0.05) (Figure 5). Anti-HNE binds poorly to MAP#15 modified with HNE (OD of 0.56 ± 0.037 compared to OD of 0.17 ± 0.08 for binding to MAP#15 not modified with HNE), indicating that this peptide is not amenable to binding to HNE, in spite of the presence of three lysines and two histidines (one of which is inaccessible). MAP#2 is not susceptible to HNE modification, since there is only one histidine which is only partially accessible (OD of 0.288 ± 0.009 for the HNE modified peptide vs 0.196 ± 0.014 for the non- HNE modified peptide). One lysine each is inaccessible in MAPs 12 and 21, but the presence of two additional lysines in each case explains the reason for the observed modification (OD of 1.81 ± 0.478 for HNE modified MAP# 12 and 1.25 for HNE modified MAP# 21) (Figure 5).

Most of the sequences that were highly modified lie in regions that are easily accessible. Exceptions to this were the MAPs 5, 6, 15, 16 and 19. Even though peptide 5 and 6 are easily accessible they were not modified by HNE in the *in vitro* assay (Figure 5). Conversely, peptide#11 (Figure 5) was highly modified but happens to be in a region that is only partially accessible (Figure 6).

4. Discussion

In an earlier study we demonstrated a novel mechanism for epitope spreading (Scofield et al, 2005). We observed accelerated acquisition of new autoreactivity upon immunization of rabbits with HNE-modified Ro60 compared to immunization with unmodified Ro60. Here, we determined the sequences on the Ro60 protein that are modified with HNE and specifically a polypeptide sequence that was susceptible for high modification with HNE for possible use as a reagent for further immunization studies.

Cysteines, lysines and histidines are the targets of oxidative modification by HNE. If all of them were to be modified, the Ro protein would have a hypothetical molecular weight of 71,400 instead of 60,000 for the unmodified protein (Kurien and Scofield, 2004). We were unable to ionize the whole Ro or HNE-modified Ro antigen by MALDI TOF and hence were unable to obtain the relative molecular weights and thus arrive at total HNE-modification in the Ro autoantigen.

In vitro HNE modification of Ro60 MAPs yielded several sequences that were highly modified. However, there were several sequences that were not modified, in spite of the fact that they contain lysines, histidines or cysteines. For example, MAP#16 had 3 lysines, while peptide #5 had a lysine and a cysteine. It is possible that the milieu in the vicinity, the local conformation of these peptides or the order in which lysines, histidines or cysteines occur may be additional contributory factors for proper HNE modification.

Human Ro60 was found to be oxidatively modified (HNE-modified) in human liver (Scofield et al, 2005), suggesting the possibility that Ro60 in lupus patients may be subject to modification, especially since we and others have found increased oxidative damage in SLE (Michel et al, 1997; Serban and Tanaseanu, 1994; Grune et al, 1997; Turi et al, 1997; Serban et al, 1996). Such a model proposes developing antibodies to Ro60 and thus autoimmunity to the entire Ro ribonucleoprotein particle after an initial immune response to oxidized Ro60. Consequently, we immunized rabbits with HNE-modified Ro60 and a similar effect was observed (Scofield et al, 2005). Distinct intra-molecular and intermolecular epitope spreading effects were seen in these animals.

We showed that some Ro-MAPs bind to the native Ro60 molecule, in our previous study (Scofield et al, 1999). The Ro-peptides that associate with the Ro60 protein could be (a) the region on the native Ro60 interacting *in vivo* with the other sequences of the same Ro60 molecule as it folds upon itself to form a stable 3-D structure, or; (b) the exposed sequences on the native Ro60 molecule interacting *in vivo* with another Ro60 molecule or molecules to form multimers. Boire and Craft (1990) have demonstrated that purified Ro RNP particles have high Stokes' radii corresponding to a globular protein with a molecular weight of 300,000 to 350,000. Since the purified Ro-ribonucleoprotein has a combined molecular weight of only about 100,000 (60,000 for the protein and 27,000 for hY5 or 37 for hY1) and has an apparent molecular weight of 60,000 on SDS-PAGE, this observation suggests that Ro particles or their individual components exist as multimers. Boire and Craft (1990) also showed that the Ro RNPs exist as three different populations, Ro^{hY5}, Ro^{hY4} and Ro^{hY1-4} in the HeLa cell, depending upon whether the hY5, hY4, or hY1 and hY3

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Figure 1:
SPR analysis of rabbit anti-Ro60 or anti-HNE Ro60 interacting with Ro-MAP-310 coupled to a sensor surfaces. Rabbits were immunized with either Ro60 or HNE-modified Ro60. Top panel- Anti-Ro60 binding to Ro-MAP-310. Bottom panel- Anti-HNE-Ro60 interacting with Ro-MAP-310.

Figure 2:

SPR analysis of rabbit anti-Ro60 or anti-HNE Ro60 interacting with Ro-MAP-190 and Ro-MAP-401 coupled to separate sensor surfaces. Rabbits were immunized with either Ro60 or HNE-modified Ro60. A- Anti-Ro60 binding to Ro-MAP-190. B- Anti-HNE-Ro interacting with Ro-MAP-190. C: Anti-Ro60 interacting with Ro-MAP-401. D: Anti-HNE-Ro interacting with Ro-MAP-401.

Figure 3:

SPR analysis of rabbit anti-Ro60 or anti-HNE Ro60 interacting with Ro-MAP-449 and Ro-MAP-482 coupled to separate sensor surfaces. Rabbits were immunized with either Ro60 or HNE-modified Ro60. A - Anti-Ro60 binding to Ro-MAP-449. B- Anti-HNE-Ro interacting with Ro-MAP-449. C: Anti-Ro60 interacting with Ro-MAP-482. D: Anti-HNE-Ro60 interacting with Ro-MAP-482.

Figure 4:

SPR analysis of the protein-protein interaction of Ro60 or HNE-Ro60 with Ro-MAP-331 and Ro-MAP-482 coupled to sensor surface. A- HNE Ro60 interacting with Ro-MAP-331. B- Ro60 interacting with Ro-MAP-331. C- HNE Ro60 interacting with Ro-MAP-482. D- Ro60 interacting with Ro-MAP-482.

Figure 5:

Binding of anti-HNE to unmodified and HNE-modified Ro60 MAPs. MAPs that were modified with HNE (post immobilization) or not modified with HNE were tested for their ability to bind anti- HNE antibodies. The MAPs are designated by their numbers assigned as given in Table 1. Values are means $\pm$ S.D. for four determinations.

Figure 6:

Crystal structure of the 60 kD Ro molecule [PDB ID: 1YVP] in worm representation. The colored portions with corresponding numbers in each case represents the sequence of each individual peptide given in Table 1. Ro60 MAPs 4, 7, 9, 10, 11, 12, 13, 19, 20, 21 were significantly modified by HNE compared to Ro60 MAPs 2, 3, 5, 6, 8, 14, 15, 16 and 17. This figure was made by the program MOLMOL (Koradi et al, 1996).

Table 1:

Ro 60 multiple antigenic peptides (MAPs), their sequence, amino acid position and molecular weight (measured by mass spectrometry)

Map #	Amino acid sequence	Location on protein	M. Wt. by mass spec. (in kD)
2	SQEGRTTKQ	81–89	9.12
3	STKQAAFKAV	106–115	9.25
4	TFIQFKKDLKES	126–137	12.7
5	MKCGMWGRA	139–147	9.2
6	MWGRALRKAIA	143–153	11.02
7	LAVTKYKQRNGWSHK	166–180	15.37
8	LRLSHLKPS	183–191	9.25
9	VTKYITKGWKEVH	198–210	13.55
10	LYKEKALS	212–219	8.45
11	TEKLLKYL	222–229	8.9
12	EAVEKVKRTKDELE	230–243	14.23
13	HLLTNHLKSKEVWKAL	257–272	16.18
14	ALLRNLGKMTA	280–290	10.34
15	NEKLLKKARIHPFH	310–323	14.69
16	YKTGHGLRGKLGKWRP	331–345	15.22
17	AAFYKTFKTV	355–364	10.25
19	MVVTRTEKDSY	401–411	11.4
20	LPMIWAQKTNTTP	449–460	12.04
21	ALREYRKKMDIPAK	482–495	14.59
22	PPPGRRPP	Sm	7.63