

**Routsias-JG; Sakarellos-Daitsiotis-M; Detsikas-E; Tzioufas-AG; Sakarellos-C; Moutsopoulos-HM** *Τα αντισώματα έναντι του EYRKKMDI (σχετιζόμενο με τον vesicular stomatitis virus πεπτίδιο) αποτελούν μόνο μια μειονότητα των συνολικών anti-Ro60kD αντισωμάτων.* Immunol. 1994 Dec; 98(3): 414-8

Προηγούμενες μελέτες έχουν υποδείξει μια πιθανή αντιγονική συσχέτιση μεταξύ του καρβοξυτελικού τμήματος του αυτοαντιγόνου Ro 60kD και της νουκλεοκαψιδικής πρωτεΐνης (N) του ιού της Φυσσαλιδώδους Στοματίτιδας (VSV). Για να ελέγξουμε εάν τα αντί-Ro/SSA αυτοαντισώματα αντιδρούν με την ομόλογη με το VSV περιοχή της πρωτεΐνης Ro 60kD, συνθέσαμε σύμφωνα με τη μέθοδο του Merrifield το οκταπεπτίδιο EYRKKMDI (8p) που εμπεριέχει την κοινή αλληλουχία αμινοξέων της πρωτεΐνης N του VSV. Ελέχθηκαν οροί 61 ασθενών με αυτοάνοσες ρευματικές παθήσεις [34 με Συστηματικό Ερυθρηματώδη Λύκο (ΣΕΛ), 21 με Σύνδρομο Sjogren's (ΣΣ) και 6 με Ρευματοειδή Αρθρίτιδα (ΡΑ)] καθώς και 59 οροί εθελοντών αιμοδοτών για την παρουσία (i) αυτοαντισωμάτων αντί-Ro 60kD με ELISA και ανοσοαποτύπωση (IB) και (ii) αντί-8p αντισωμάτων με ELISA. Ανεβρέθηκαν αντισώματα κατά του 8p στους 9/31 των θετικών στο IB αντί-Ro 60kD ορών, 5/30 των αρνητικών στο IB ορών και 2/59 των φυσιολογικών ορών ελέγχου. Η συμφωνία μεταξύ της ELISA αντί-8p και του IB αντί-Ro 60kD ήταν χαμηλή ( $\chi^2=0,71$ ,  $p=0.4$ ) σε αντίθεση με τη ELISA αντί-Ro 60kD και του IB αντί-Ro 60kD ( $\chi^2=27,6$ ,  $p=10^{-7}$ ). Επιπλέον καθαρισμός με ανοσοσυγγένεια των αντί-8p αντισωμάτων από έναν ισχυρά θετικό αντί-8p και αντί-Ro 60kD ΣΕΛ ορό οδήγησε σε εξάλειψη της αντί-8p δραστηριότητας κατά 95% ενώ η αντί-Ro 60kD δραστηριότητα του μειώθηκε κατά 37%. Πειράματα αναστολής με τα καθαρισμένα αντισώματα αντί-8p έδειξαν ότι με το οκταπεπτίδιο μπορούσε να επιτευχθεί αναστολή της αντί-Ro 60kD δραστηριότητας τους κατά 94,5% ενώ με τη Ro 60kD πρωτεΐνη είχαμε αναστολή της αντί-8p δραστηριότητας τους κατά 42,3%. Προεπώαση του ορού με το οκταπεπτίδιο έδωσε αναστολή 4% στη ELISA αντί-Ro 60kD.

Τα αποτελέσματα αυτά υποδεικνύουν ότι τα αντί-8p αντισώματα (αντισώματα κατά της ομόλογης περιοχής του VSV στην Ro 60kD πρωτεΐνη) είναι μόνο ένα μικρό μέρος των αντί-Ro αυτό-αντισωμάτων και ο ιός VSV δεν φαίνεται να ευθύνεται για την γένεση της αντί-Ro αυτοαντισωματικής απόκρισης.

## Antibodies to EYRKK vesicular stomatitis virus-related peptide account only for a minority of anti-Ro60kD antibodies

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(Accepted for publication 16 August 1994)

### SUMMARY

Previous studies demonstrated a possible antigenic relation between the carboxyl terminal portion of anti-Ro60kD autoantigen and a nucleocapsid protein (N) of vesicular stomatitis virus (VSV). In order to investigate whether anti-Ro60kD autoantibodies react with the VSV homologous region of the Ro60kD protein we synthesized, according to Merrifield's method, the EYRKKMDI octapeptide (8p) sharing a common sequence with the N protein of VSV. Sera from 61 patients with autoimmune rheumatic diseases (34 systemic lupus erythematosus (SLE), 21 Sjögren's syndrome (SS) and six rheumatoid arthritis (RA)) as well as 59 from normal blood donors were tested for the presence of anti-Ro60kD autoantibodies by ELISA and immunoblot (IB) and anti-8p antibodies by ELISA. Antibodies to 8p were found in 9/31 of anti-Ro60kD IB-positive sera, 5/30 of anti-Ro60kD-negative sera and 2/59 of normal control sera. The concordance between the anti-8p ELISA and the anti-Ro60kD IB was very poor ( $\chi^2 = 0.71$ ,  $P = 0.4$ ) in contrast to the anti-Ro60kD ELISA and the anti-Ro IB ( $\chi^2 = 27.6$ ,  $P = 10^{-7}$ ). Subsequent affinity purification of the anti-8p antibodies from a strong positive anti-8p and anti-Ro60kD SLE serum yielded 95% depletion of the anti-8p activity and 37% reduction of the anti-Ro60kD activity. Inhibition assays with the affinity-purified anti-8p antibodies demonstrated that the octapeptide gave 94.5% inhibition of the anti-Ro60kD activity, while Ro60kD protein led to 42.3% inhibition of the anti-8p. Preincubation of the serum with the octapeptide produced 4% inhibition of anti-Ro60kD ELISA. These results indicate that the anti-8p antibodies account only for a minority of the anti-Ro60kD autoantibodies.

**Keywords** vesicular stomatitis virus autoimmune rheumatic diseases synthetic peptides Ro/SSA systemic lupus erythematosus

### INTRODUCTION

Sera from patients with autoimmune rheumatic diseases including Sjögren's syndrome (SS), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) often contain circulating antibodies directed against the Ro/SSA autoantigen [1–3]. Ro/SSA is an RNA–protein complex (RNP) composed of proteins non-covalently associated with a family of small cytoplasmic RNAs called hY1, hY3, hY4 and hY5 RNAs [4]. The protein component of Ro/SSA RNP consists of at least three immunologically distinct proteins, namely: 60-kD Ro/SSA, 52-kD Ro/SSA and La/SSB protein [5]. The biological function of Ro RNP remains unknown. Antibodies to Ro autoantigen are

primarily directed against the protein components of Ro RNP [6], although the antigenic determinants (B cell epitopes) are not well understood. On clinical grounds the presence of anti-Ro60kD autoantibodies is strongly associated with the pathogenesis of neonatal lupus, complete congenital heart block, and subacute cutaneous lupus [7,8].

Recently Scofield *et al.* [9], using proteolytic digestion of bovine Ro60kD, identified a major immunoreactive fragment (13 kD) of Ro60kD localized at the carboxyl end of the molecule. A panel of sera, tested against overlapping synthetic octapeptides covering the 13-kD segment of Ro60kD, revealed a binding pattern quite varied among the anti-13-kD positive sera. The 485–492 (EYRKKMDI) sequence was defined as one of the major antigenic determinants of Ro60kD autoantigen, and a closely related EYRKKLMD segment was found in the nucleocapsid (N) protein of vesicular stomatitis virus (VSV). In

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a subsequent study it was demonstrated that anti-Ro60kD precipitin-positive sera recognize at least 19 synthetic peptide groups of the entire Ro protein, and the 13-kD sequence shares two of them, including the VSV homologous region [10,11]. Thus, it was proposed that the autoimmune response to Ro/SSA is related to the immune processing of the N protein of VSV [12,13].

The present study was designed to investigate the relationship between the autoimmune response to Ro/SSA and the immune processing of the N protein of VSV. We report on both the synthesis and the immunological characteristics of EYRKKMDI synthetic octapeptide (485–492) of Ro/SSA, which shares an homologous sequence (EYRKK) with the EYRKKLMD (151–158) segment of the N protein of VSV.

## PATIENTS AND METHODS

### Sera

Thirty-four sera from patients with SLE, 21 with SS and six with RA were collected from patients fulfilling the revised criteria for the classification of SLE [14], SS [15] and RA [16]. Thirty-one out of 61 sera were found positive for anti-Ro/60kD autoantibodies, by immunoblot. Fifty-nine normal sera were collected from healthy blood donors and used as normal controls.

### Peptide synthesis and purification of the EYRKKMDI octapeptide (8p)

Solid-phase synthesis of the EYRKKMDI (Glu<sup>485</sup>-Tyr-Arg-Lys-Lys-Met-Asp-Ile<sup>492</sup>) using N<sup>α</sup>-t-Boc-L-isoleucine-PAM resin (1% cross-linked divinyl-benzene-styrene, 1.2 g, 0.6 mmol/g) anchor bond and N<sup>α</sup>-t-Boc/benzyl-side chain protection was carried out by standard methods [17]. Amino acid couplings were performed by the dicyclohexyl carbodiimide (DCC)/hydroxybenzotriazole (HOBt) procedure using a ratio of amino acid/DCC/HOBt/resin (3/3/3/1). Deprotection of the N<sup>α</sup>-t-Boc protecting group was carried out using trifluoroacetic acid (TFA) followed by diisopropylethylamine for neutralization. After synthesis, the peptide was cleaved from the resin with anhydrous HF in the presence of anisole and phenol (10% v/v) as scavengers at 0°C for 1 h. The peptide was extracted from the resin with 2 M acetic acid and lyophilized to give 0.74 g of the 8p (95% yield). Purification of the crude peptide was achieved by partition chromatography on Sephadex G-25 in But/Pyr/AcOH/H<sub>2</sub>O (5/5/1/4 v/v). The peptide was homogeneous as determined by thin layer chromatography in But/Pyr/AcOH/H<sub>2</sub>O (5/5/1/4 v/v) (R<sub>f</sub> = 0.41) and AcOH 2N (R<sub>f</sub> = 0.53). The overall yield after purification was 0.56 g (76% yield). Appropriate 1-D and 2-D <sup>1</sup>H-nuclear magnetic resonance (NMR) spectra confirmed the identity and the purity of the peptide.

### Purification of Ro/SSA protein

Ro/SSA 60-kD protein was purified according to a previously described method [18]. Briefly, human spleen was homogenized in an equal amount (w/v) of PBS pH 7.2. The cell lysate was centrifuged at 10000 g for 1 h and the supernatant was mixed with 40% (v/v) DE-52 overnight followed by extraction with 0.5 NaCl in PBS pH 7.2. The extract was then applied to an anti-Ro/SSA 60-kD affinity column. The column was made by coupling purified IgG from SLE monospecific serum to cyanogen bromide

(CNBr)-activated Sepharose 4B using conventional methods. Ro/SSA was eluted from the affinity column with HCl-Glycine pH 2.7 and the eluates were dialysed against PBS pH 7.2. The preparation was evaluated by SDS gel electrophoresis and found to contain essentially the Ro60kD protein.

### Cell extract and immunoblotting

Cytoplasmic extract was prepared from cultured HeLa cells as described by Habets *et al.* [19]. Samples of cytoplasmic extracts corresponding to 50 × 10<sup>6</sup> cells were analysed using 10% PAGE after boiling in SDS (4%) and 2-mercaptoethanol (2-ME; 10%). SDS-PAGE gel electroblotted on nitrocellulose membranes [20]. Nitrocellulose blots after staining with Ponceau 5 were cut into strips. The blotted strips were blocked with non-fat milk 5% in TBS and incubated with diluted sera (1 : 50) at 4°C overnight. Afterwards a solution of goat anti-human IgG conjugated to peroxidase was added and allowed to react for 1 h. The colour was developed by adding a substrate solution of 4-chloro-1-naphthol to the strips.

### Anti-8p antibodies purification

An anti-Ro60kD strongly positive serum was selected for its capacity to recognize the 8p. IgG from this serum, purified by protein A Sepharose column, was concentrated and dialysed against PBS. The 8p was then covalently attached to CNBr-activated Sepharose 4B and the purified IgG was passed through. The column was washed with PBS, eluted by changing the pH, and the eluates were neutralized, concentrated and dialysed against PBS. IgG concentrations were evaluated by nephelometry (Beckman Instruments, Brea, CA).

### ELISA

The levels of anti-Ro/SSA antibodies were determined by commercially available ELISA (Diastat anti-Ro; Shield Diagnostics, London, UK) using microtitre wells coated with affinity-purified bovine Ro/SSA antigen, according to the manufacturer's instructions.

A modified solid-phase assay (ELISA) for anti-8p antibodies was also applied [21]. Nunc microtitre plates were coated with 50 μl of 8p (100 μg/ml) in ethanol overnight at 4°C. Our preliminary studies demonstrated that 5 μg/well of the peptide was the optimal concentration for detection of 7.5 μg/well IgG antibodies. After blocking the remaining binding sites with 1% bovine serum albumin (BSA), 1% ovalbumin and 0.1% Tween 20 in PBS for 1 h at room temperature, the plates were incubated with IgG (150 μg/ml) or diluted sera (1 : 50 in blocking buffer) for 3 h. The wells were then washed (PBS Tween 20) and goat anti-human IgG (0.5 mg/ml) conjugated to alkaline phosphatase (1 : 1500 in blocking buffer) was added. The plates were incubated for 1 h, washed as previously, and *p*-nitrophenyl phosphate substrate was added. Colour development was read at 450 nm with a microELISA reader (Dynatech-UK). The reliability of anti-8p ELISA was tested as follows: (i) IgG from all patient sera was measured by nephelometry (mean 1580 ± 420 mg/dl). No correlation between IgG levels and ELISA OD values was observed (*P* > 0.1); (ii) all sera were tested against BSA or RLGRG (control peptide) according to the precited method. Normal and patient sera showed very low and almost identical levels in both BSA and control peptide ELISA; (iii) anti-8p depletion of an anti-VSV strongly positive serum yielded 95% reduction of

the optical density in anti-8p ELISA; (iv) 8p caused 55.8% homologous inhibition in the binding of the anti-8p antibodies in ELISA.

ELISA values were expressed as OD units, according to the formula:

$$\text{OD unit} = \frac{\text{OD serum tested}}{\text{OD normals} + 2 \text{ s.d.}} \times 100$$

#### *Inhibition assays*

IgG samples taken from the previously mentioned strongly positive serum were incubated with the soluble EYRKKMDI peptide (concentrations from 25 µg/ml to 2 mg/ml) or purified human Ro60kD antigen (10 µg/ml) for 2 h at room temperature before application in the ELISA test.

#### *Statistical analysis*

Correlations between the anti-Ro60kD and anti-8p antibodies were analysed using the  $\chi^2$  test (with Yates' correction). Student's *t*-test was used for mean OD comparisons of anti-Ro60kD and anti-8p binding levels.

## RESULTS

#### *Relationship of the anti-Ro60kD to the anti-8p antibodies*

Human autoimmune sera were tested in immunoblot for antibodies against the denatured form of Ro60kD. The same sera were evaluated in ELISA to quantify anti-Ro60kD and anti-8p antibodies. Twenty-six of the 31 Ro60kD immunoblot-positive sera were also positive in anti-Ro60kD ELISA, while 9/31 were positive in anti-8p ELISA. The antibody binding levels of anti-Ro/SSA sera were higher in anti-Ro60kD ELISA compared with the anti-8p ELISA ( $t = 5.46$ ,  $P = 9 \times 10^{-7}$ ). Anti-Ro60kD immunoblot-negative sera were found positive in anti-Ro60kD (4/30) and anti-8p (5/30) ELISA. The concordance of the anti-Ro60kD immunoblot and anti-Ro60kD ELISA experiments was very high ( $\chi^2 = 27.59$ ,  $P \approx 10^{-7}$ ), in contrast to the anti-Ro60kD immunoblot and anti-8p ELISA ( $\chi^2 = 0.71$ ,  $P = 0.4$ ).

Anti-Ro60kD antibodies were found in 13/34 SLE sera, 13/21 SS sera and 4/6 of RA sera, while anti-8p antibodies were found in 9/34 SLE sera, 5/21 SS sera and 0/6 RA sera (Fig. 1).

#### *Anti-Ro60kD reactivity of the anti-8p antibodies*

IgG from one SLE serum, which gave strong positive reaction in both anti-Ro60kD and anti-8p ELISA, was purified by the protein A-Sepharose method and then passed through an 8p-coupled Sepharose column. Elution of the anti-8p antibodies, performed by changing the pH, yielded two major IgG fractions at pH 4.5 (A) and 2.7 (B) (Fig. 2). IgG from the initial SLE sample before and after treatment with the 8p column, as well as the IgG elution fractions, were tested at the same concentrations in anti-Ro60kD and anti-8p ELISA. Both elution fractions possessed 36% and 300% of the initial anti-Ro60kD and anti-8p reactivity, respectively. In contrast, the sample after treatment with the 8p column preserved 73% of anti-Ro60kD reactivity and only 5% of the anti-8p reactivity (Fig. 2).

#### *Inhibition studies on the anti-8p antibodies*

Cross-inhibition assays demonstrated that 8p inhibited the

anti-Ro60kD reactivity of (i) the elution fractions A and B, (ii) IgG from SLE before treatment with the 8p column, and (iii) IgG pretreated with the 8p column at about 50.2%, 94.5%, 4.9% and 3.9%, respectively. In addition, preincubation of the anti-8p antibodies (elution fraction B) with purified Ro/SSA autoantigen resulted in 68% inhibition of anti-Ro60kD reactivity and 44% inhibition of anti-8p reactivity. Finally, homologous inhibition assay showed that 8p caused 55.8% inhibition of the anti-8p antibody activity.

## DISCUSSION

Molecular mimicry as a mechanism for the induction and perpetuation of autoimmunity has been the subject of great research interest [22]. One of the well documented examples of molecular mimicry between infective agents and autoantigens is the gp120 of HIV and HLA class II antigens [23]. However, it is likely that many of the sequence similarities reported in the last decade occur by chance, and the potentially cross-reactive sequences are in fact repeat sequences. In previous studies [9–13] it was proposed that the anti-Ro60kD autoimmune response is related to the immune processing of a nucleocapsid protein (N) of VSV due to sequence similarities between the 485–492 sequence of Ro60kD (an antigenic determinant according to Scofield *et al.* [9]) and the 151–158 sequence of the N protein in VSV.

A panel of 61 sera from patients with autoimmune rheumatic diseases was screened, initially on electroblots from SDS gels, for the presence of anti-Ro60kD antibodies. These sera were screened for antibodies against the denatured form of Ro60kD, which are more likely to bind linear epitopes [24,25]. Our results indicate a poor concordance between anti-Ro60kD immunoblot and anti-8p ELISA, contrary to the anti-Ro60kD immunoblot and anti-Ro60kD ELISA.

In a previous study [12] it was shown that anti-VSV (anti-nucleocapsid protein) antibodies were found more frequently in anti-Ro60kD-positive SLE sera (41.5%) than in anti-Ro60kD-negative SLE sera (20.5%). In our study the prevalence of antibodies to 8p was not statistically different between anti-Ro60kD-positive and -negative SLE sera (33.3% and 21.1%, respectively).

Although the anti-8p immune response was not associated with the anti-Ro60kD reactivity, correlation between them was observed in the case of one SLE serum, which gave strong positive reaction for both anti-Ro60kD and anti-8p ELISA. Purified IgG from the reported SLE serum, which passed through an 8p-coupled Sepharose column, resulted in depletion of the anti-8p reactivity, while the anti-Ro60kD reactivity was maintained. Interestingly the IgG eluents from this column preserved 300% of the anti-8p reactivity and only 36% of the anti-Ro60kD reactivity, when examined at the same initial IgG concentration. These findings suggest that anti-8p antibodies represent a limited minority of the anti-Ro60kD antibodies. Alternatively, it is possible that anti-8p antibodies are completely distinct from anti-Ro60kD antibodies, since short peptide moieties may exist in various flexible conformations, compared with the more rigid homologous region of Ro60kD protein [26]. In this case the observed anti-Ro60kD reactivity could be attributed to the ability of some anti-Ro60kD antibodies to bind IgG [27].

In order to distinguish these possible explanations, IgG

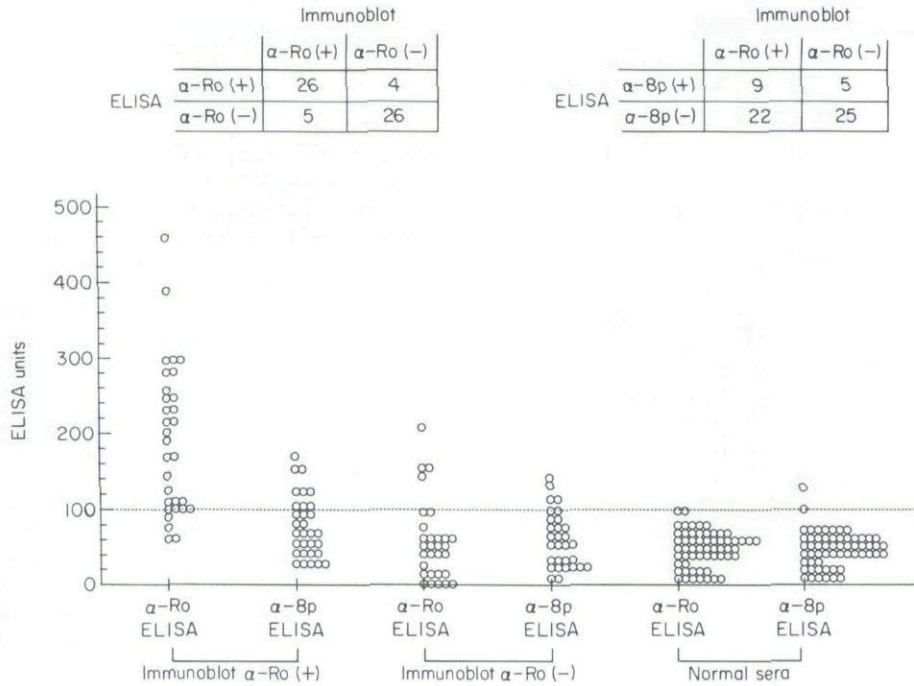


Fig. 1. Binding of anti-Ro and anti-8p ELISA for immunoblot anti-Ro positive, anti-Ro negative and normal sera. Dotted line represents 2 s.d. above the mean binding levels of normal sera.

samples were applied in homologous and cross-inhibition assays. The 8p induced inhibition of binding of anti-8p antibodies in both Ro and 8p ELISA assays. Preincubation of anti-8p antibodies with Ro antigen resulted in considerable inhibition of both anti-Ro and anti-8p reactivity. These results indicate that some anti-Ro antibodies react actually with the VSV homologous region on Ro protein. It should also be mentioned that IgG anti-8p elution fractions had different properties in regard to Ro60kD and 8p binding. In fact, 8p induced 94.5% (elution fraction B) and 50.2% (elution fraction A) inhibition of anti-Ro60kD reactivity. Taking into consideration the different pH levels used for the IgG elution (pH 4.2 and 2.7 for fractions A and B, respectively) it is very possible that autoantibodies eluted at higher pH possess low affinity to the VSV<sub>pep</sub> and high affinity to Ro60kD antigen. In contrast, antibodies eluted at lower pH showed high affinity to both 8p and Ro60kD antigen. Moreover, preincubation of the initial IgG preparation with the 8p yielded approximately 5% inhibition in anti-Ro60kD ELISA, which further confirms that anti-8p antibodies represent a minority of anti-Ro60kD antibodies.

Taking into account the reported results we can conclude that anti-8p antibodies were not frequently found in sera from patients with autoimmune rheumatic diseases and anti-Ro60kD antibodies. However, the existence of anti-8p-specific antibodies among the anti-Ro60kD antibodies is not surprising, since it is known that the anti-Ro/SSA autoimmune response is characterized by its heterogeneity. Several anti-Ro/SSA subpopulations have been reported to be associated with particular diseases, and conformational diversity is dependent on various molecular forms of Ro/SSA antigen [24,28,29]. These findings are in agreement with previous reports, which failed to demonstrate the presence of main B cell epitopes on the carboxy terminus of Ro60kD autoantigen, where the sequence EYRKK is located [30]. They are also consistent with our recent results, which disclose two major disease-specific anti-Ro60kD targets on the central region of Ro60kD [31].

It is also interesting to note that although VSV human infection has not been documented in Europe, anti-Ro/SSA antibodies and the related autoimmune rheumatic diseases are found throughout the world. Consequently, VSV cannot be

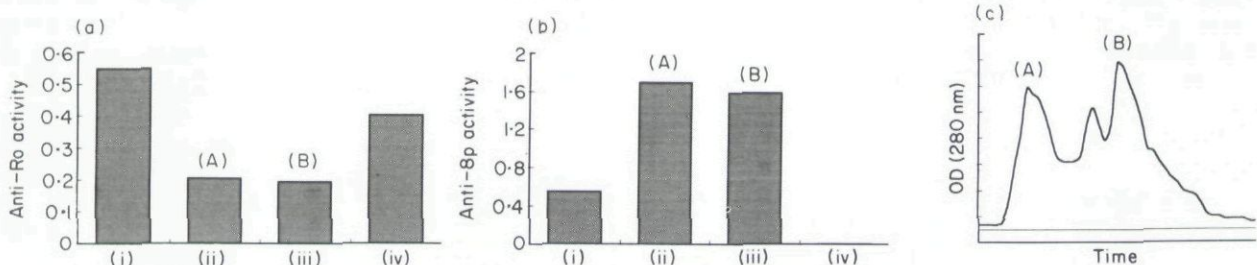


Fig. 2. Anti-Ro60kD (a) and anti-8p (b) binding capacity for total IgG (i), anti-8p IgG of elution fractions A (ii) and B (iii), and IgG after anti-8p depletion (iv). (c) Absorbance of anti-8p eluates at 280 nm.

itself responsible for anti-Ro60kD autoantibodies and autoimmune diseases. On the other hand, anti-8p antibodies do exist, even as a minority of the anti-Ro60kD response, and their diagnostic and biological significance remains to be further investigated.

#### ACKNOWLEDGMENT

The authors thank the E.U. for financial support.

#### REFERENCES

- Moutsopoulos HM, Zerva LV. Anti Ro(SSA)/La (SSB) antibodies and Sjögren's syndrome. *Clin Rheumatol* 1990; **9** (Suppl. 1): 1-9.
- Reichlin M, Harley JB. Antibodies to Ro/SSA and the heterogeneity of systemic Lupus Erythematosus. *J Rheumatol* 1987; **14** (Suppl. 13): 112-117.
- Skopouli FN, Andonopoulos AP, Moutsopoulos HM. Clinical implications of the presence of anti-Ro(SSA), antibodies in patients with rheumatoid arthritis. *J Autoimmun* 1988; **3**:381-8.
- Pruijn GJ, Slobbe RL, Van Venrooij WJ. Structure and function of La and Ro RNPs. *Mol Biol Reports* 1990; **14**:43-48.
- Slobbe RL, Pruijn GJ, Van Venrooij WJ. Ro(SS-A) and La(SS-B) ribonucleoprotein complexes: structure function and antigenicity. *Ann Med Intern* 1991; **42**:592-600.
- Wolin SL, Steitz JA. The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding site on the Ro RNAs. *Proc Natl Acad Sci USA* 1984; **81**:1996-200.
- Buyon JP, Ben-Chetrit E, Karp S *et al*. Acquired congenital heart block. Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J Clin Invest* 1989; **84**:627-34.
- Lee LA, Gaither KK, Coulter SN, Norris DA, Harley JB. Pattern of cutaneous immunoglobulin G. Deposition in subacute cutaneous lupus erythematosus is reproduced by infusing purified anti-Ro(SSA) autoantibodies into human skin-grafted mice. *J Clin Invest* 1989; **83**:1556-62.
- Scofield RH, Dickey WD, Jackson KW, James JA, Harley JB. A common autoepitope near the carboxyl terminus of the 60-kD Ro ribonucleoprotein: sequence similarity with a viral protein. *J Clin Immunol* 1991; **11**:378-88.
- Scofield RH, Harley JB. Autoantigenicity of Ro/SSA antigen is related to a nucleocapsid protein of vesicular stomatitis virus. *Proc Natl Acad Sci USA* 1991; **88**:3343-7.
- Huang SC, Yu H, Barler BD, Scofield RH, Harley JB. Linear epitopes of Ro. 27th ACR Annual Meeting. Atlanta, USA 12-17 Oct. 1992; *Arthritis Rheum* 1992; **35**:S171 (abst.).
- Hardgrave KL, Neas B, Scofield RH, Harley JB. Antibodies to vesicular stomatitis virus proteins in patients with systemic lupus erythematosus and in normal subjects. *Arthritis Rheum* 1993; **36**:962-70.
- Hardgrave KL, Neas B, Scofield RH, Harley JB. Antibodies binding vesicular stomatitis virus proteins in systemic lupus erythematosus patients and normals. 27th ACR Annual Meeting. Atlanta, USA 12-17 Oct. 1992; A259. *Arthritis Rheum* 1992; **35**:S112 (Abstr.).
- Tan EM, Cohen AS, Fries JB. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25**:1271-7.
- Vitali C, Bombardieri S, Moutsopoulos HM *et al*. Preliminary criteria for the classification of Sjögren's syndrome. *Arthritis Rheum* 1993; **36**:340-7.
- Arnett FC, Edworthy SM, Bloch DA *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**:315-24.
- Merrifield RB. Solid phase peptide synthesis I. The synthesis of a tetrapeptide. *J Am Chem Soc* 1963; **85**:2149-54.
- Mamula MJ, O'Brien CA, Harley JB, Hardin JA. The Ro ribonucleoprotein particle: induction of autoantibodies and the detection of Ro RNAs among species. *Clin Immunol Immunopathol* 1989; **52**:435-46.
- Habets WJ, De Rooij DJ, Hoet MH, Van de Putte LB, Van Venrooij WJ. Quantitation of anti-RNP and anti-Sm antibodies in MCTD and SLE patient by immunoblotting. *Clin Exp Immunol* 1985; **59**:457-66.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**:4350-4.
- Engvall E, Perlman A. Enzyme-linked immunosorbent assay (ELISA) quantitative assay for immunoglobulin G. *Immunochemistry* 1971; **8**:871-4.
- Horsfal AC. Molecular mimicry and autoantigens in connective tissue diseases. *Mol Biol Rep* 1992; **16**:139-47.
- Garry RF. Extensive antigenic mimicry by retrovirus capsid proteins. *AIDS Res Hum Retroviruses* 1990; **6**:1361-2.
- Boire G, Lopez-Longo FJ, Laporte S, Menand HA. Sera from patients with autoimmune disease recognize conformational determinants on the 60-kD Ro/SS-A protein. *Arthritis Rheum* 1991; **34**:722-30.
- Geysen HM, Rodda SJ, Mason TJ, Tribbick G, Schoofs PG. Strategies for epitope analysis using peptide synthesis. *J Immunol Methods* 1987; **102**:259-74.
- Kaiser ET, Kerdy FJ. Secondary structures of proteins and peptides in amphiphilic environments (A review). *Proc Natl Acad Sci USA* 1983; **80**:1137-43.
- Mamula MJ, Fax OF, Yamagata H, Harley JB. The Ro/SSA autoantigen as an immunogen. Some anti-Ro/SSA antibody binds IgG. *J Exp Med* 1986; **164**:1889-901.
- Boire G, Craft J. Biochemical and immunological heterogeneity of the Ro ribonucleoprotein particles. Analysis of sera specific for the Ro hY5 particle. *J Clin Invest* 1989; **84**:270-9.
- Barakat S, Meyer O, Torterotot F, Youinou P, Brand JP, Kahn MF, Muller S. IgG antibodies from patients with primary Sjögren's syndrome and systemic lupus erythematosus recognize different epitopes in 60-kD SSA/Ro protein. *Clin Exp Immunol* 1992; **89**:38-45.
- Wahren ME, Ryden U, Andersson B, Ringertz NR, Petterson I. Characterization of autoepitopes present on the human SSA/Ro 60kD protein using recombinant antigen and synthetic peptide. *Clin Exp Rheum* 1991; **9**:332 (Abst.).
- Routsias JG, Tzioufas AG, Sakarellos C, Sakarellos-Daitsiotis M, Moutsopoulos HM. Preferred antibody recognition of the 169-190 and 211-232 sequences of the Ro60kD antigen. *Clin Rheumatol* 1994; **13**:146 (Abst.).