Peptides 1992

Proceedings of the Twenty-Second European Peptide Symposium
September 13–19, 1992, Interlaken, Switzerland

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ESCOM ▪ Leiden ▪ 1993

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Immunological studies of synthetic peptides related to the Sjögren's Syndrome

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Introduction

The Ro/SSA antigen is a common target of an autoimmune response in Systemic Lupus Erythematosus (SLE) and Sjögren's Syndrome (SS) [1]. Synthetic peptides corresponding to the amino terminal sequence of the 60 kDa Ro/SSA protein, as well as the isolated protein, were selectively recognized by anti-Ro/SSA monospecific sera. However, isolation of a cDNA encoding the 60 kDa protein revealed that this was human calreticulin [2], which did not interact with the anti-Ro/SSA monospecific sera, although it was reactive to specificities such as anti-Ro/SSA and anti-Sm and/or anti-nRNP. The present study was designed to investigate if sera from patients with rheumatic diseases recognized calreticulin (CaR), as well as to gain further insight into autoimmunity. We report on both the synthesis and immunological examination of two peptides homologous to the amino terminal of CaR and corresponding to the 1–24 (SP24) and 7–24 (SP1-8) amino acid sequence of the protein: Glu[1]-Pro-Ala-Val-Tyr-Phe-Lys[7]-Glu-Gln-Phe-Leu-Asp-Gly-Asp-Gly-Trp-Thr-Ser-Arg-Trp-Ile-Glu-Ser-Lys[24].

Results and Discussion

The CaR-peptide analogues were prepared by solid phase methodology using N^{α}-t-Boc-N^{ε}-(2-chloro-CBz)-L-Lys-PAM resin anchor-bond and N^{α}-t-Boc/benzyl side-chain protection was carried out by standard methods. The peptides were cleaved from the resin with anhydrous HF and purification was achieved by anion exchange chromatography on diethyl amino ethyl resin, using as eluant gradient of NH_3 0.1 M/H_2O. Appropriate 1D and 2D ^1H NMR spectra confirmed the identity of the peptides.

The peptides reacted in ELISA with sera from patients with SLE, SS, RA (Rheumatoid Arthritis), as well as MCTD (Mixed Connective Tissue Disease) (Fig. 1A), which, in agreement with previous observations, suggested that antiCaR autoantibodies are not restricted to any subset of the disease. Both the SP18 and SP24 peptides were positive (ELISA) to specific human sera antibodies, such as anti-Ro/SSA, anti-Ro/SSA and anti-La/SSB and in some cases with anti-nRNP (Fig. 1B) and anti-Sm (not shown in Fig. 1B), although the CaR encoded from cDNA did not exert similar reactivity. These conflicting findings may be attributed to the addi-

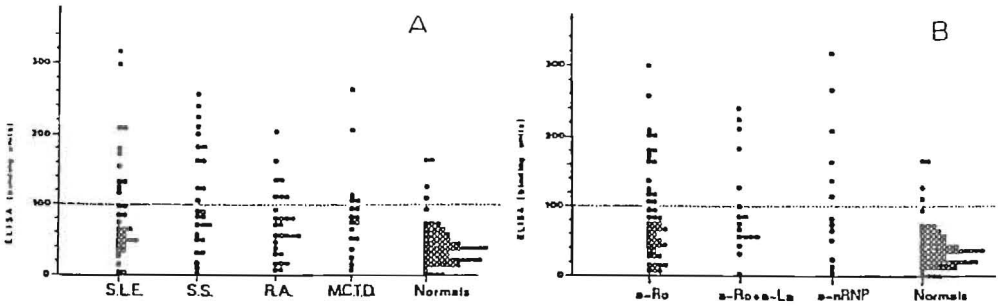

Fig. 1. Binding of sera from patients with SLE, SS, RA and NCTD (A) and a-Ro, a-Ro+a-La, and a-nRNP autoantibody specificities (B) to the SP24 CaR analogue. Dotted line represents 3 standard deviations above the mean binding levels of normal control sera.

tional hydrophobic amino terminal residues of the recombinant CaR, absent in the authentic protein, which may influence the conformational mobility of the N-terminal portion of the molecule, and consequently may affect antibody recognition. On the other hand, the CaR analogues may be more easily recognized in the anti-Ro/SSA autoimmune sera, compared to the whole protein, as a consequence of the significant degree of conformational freedom of the relatively short synthetic peptide moieties.

Even though CaR is not recognized by mono-specific anti-Ro/SSA sera, this protein is a human autoantigen. Our results argue in favor of this observation, since antibodies against the CaR analogues were detected in all the autoimmune rheumatic diseases (Fig. 1B). It was also proposed that CaR is a heat shock/stress protein and, therefore, may be related to the autoimmune mechanism. Thus, it is not surprising that anti-peptide antibodies were detected in autoimmune sera (Fig. 1B). Taking into consideration the heterogeneous nature of the Ro/SSA-nRNP particle, one could pose the question whether CaR is a component of the ribonucleoprotein complex. Identification and synthesis of CaR epitope(s) could answer the above question and provide more insight on the cellular function(s) of CaR.

References

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