Structural analysis of the major ribosomal P epitope C22

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INTRODUCTION
Autoantibodies to the three ribosomal phospho-(-P) proteins (P0, P1 and P2) summarized as Rib-P are found in 10-40% patients with systemic lupus erythematosus (SLE). The Rib-P antigen consists of three components of the 60S ribosomal subunit designated P0 (38 kD), P1 (19 kD) and P2 (17 kD). A pentameric complex of one copy of P0 and two copies each of P1 and P2 interacts with the 28S rRNA molecule forming a GTPase domain, which is active during the elongation step of protein translation. A common major epitope (C22) of the Rib P antigens has been localized to the C-term. In a more precise study the epitope-core has been identified as GFGLFD, wherein the phenylalanine residues Phe111 and Phe114 (of human P2) represent the key residues for antibody recognition.

MATERIALS AND METHODS
Membrane bound peptides prepared by SPOT technology were used to confirm the key amino acid residues of the C22 peptide recognized by anti-ribosomal P protein antibodies in a larger group of anti-Rib-P samples (n=20) all from SLE patients. Structural analysis of the C22 peptide was done by Nuclear magnetic resonance (NMR) on a Bruker DRX600 spectrometer (Bruker, Billerica, MA, USA). NMR data was used to generate an approximated molecular model of the six C-terminal amino acids using a molecular visualization and analysis program for the display and manipulation of the surfaces of molecules and their electrostatic properties (GRASP).

RESULTS
SPOT technology showed that all 20 of the anti-Rib-P positive sera recognized an epitope clustered around the amino acids Phe111 and Phe114. Based on the distance of the key amino acid residues identified by mutational analysis we suggested an alpha-helical structure of the epitope. Using NMR analysis we found that the C-terminal six amino acids of the C22 peptide show a helical tendency. Based on the NMR data we propose a 3D-model of the Rib-P major epitope showing an alpha-helical structure.

CONCLUSION
Based on mutational analysis and NMR analysis, we propose an alpha-helical structure of the major ribosomal P protein epitope. Furthermore, we conclude that the key amino acids of the epitope core are located on the same side of the alpha-helix.

REFERENCES