

## **A Point Mutation in the SSA/Ro60 Autoantigen Which Prevents Y RNA Binding Attenuates a Requisite Signal for Cell Surface Expression and TLR-Dependent Inflammation**

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### **Background/Purpose:**

The Ro/SSA ribonucleoprotein particle is a major autoantigen in Sjögren's syndrome, Systemic Lupus Erythematosus, and asymptomatic mothers of children with heart block. The particle comprises a 60kD Ro protein (Ro60) that binds misfolded RNAs in the nuclei and noncoding RNAs, Y RNAs, in the cytoplasm. During apoptosis, Ro60 translocates to the cell surface where it is available to bind extracellular antibody. Previous studies have demonstrated that the immune complex of RNA and Ro60 bound by anti-Ro60 antibody gains access to the macrophage endosome via FcγR uptake with subsequent ligation of Toll-like receptors (TLR) and secretion of proinflammatory cytokines. This study employed cells expressing novel Ro60 mutants that influence RNA binding to address the dependency of Ro60 associated RNA in translocation of Ro60 during apoptosis and activation of macrophages.

### **Method:**

Murine fibroblast cell lines were obtained in which constitutively transfected constructs of FLAG-tagged forms of mutated Ro60 were introduced into Ro60 knockout cells. Ro60 K170A, R174A (170/4) does not bind misfolded pre5S RNA but binds Y RNA. Ro60 H187S does not bind Y RNA. Evaluations included the capacity of fibroblasts either permeabilized or rendered apoptotic by staurosporine or loss of anchorage signals, to form immune complexes with anti-Ro60 antibody (flow cytometry). Function was assessed by TNFα secretion (ELISA) by macrophages obtained from human peripheral blood mononuclear cells and PMA-differentiated THP-1.

### **Result:**

Early apoptotic fibroblasts (Annexin V-positive, PI-negative) transfected with Ro60 170/4 (binds pre5S but not Y RNA) were bound by anti-Ro60, affinity purified from the serum of a mother with a child effected by heart block, but not control IgG ( $190 \pm 108$  vs  $10 \pm 9$ ,  $p=0.027$ ). Cell surface expression of this Ro60 mutant was similar to wild type Ro60 ( $348 \pm 158$ ,  $p=0.49$  vs Ro60 170/4). In contrast, apoptotic fibroblasts transfected with Ro60 H187S (does not bind Y RNA) were not bound by anti-Ro60 ( $14 \pm 8$  vs 170/4,  $p=0.029$ , vs wild type,  $p=0.029$ ). Despite differences in cell surface translocation and RNA content, both Ro60 mutants, H187S and 170/4 showed equivalent intracellular binding of anti-Ro60 ( $1345 \pm 738$  and  $1384 \pm 282$ ). The functional consequences of surface bound Ro60-anti-Ro60 complexes were subsequently addressed. Macrophages cultured with Ro60 170/4 apoptotic fibroblasts preincubated with anti-Ro60, secreted significantly higher levels of TNFα compared to macrophages incubated with Ro60 H187S apoptotic fibroblasts also preincubated with anti-Ro60 ( $146 \pm 32$  pg/ml vs  $46 \pm 14$  pg/ml respectively,  $p=0.04$ ). The TNFα secretion induced after macrophages were cultured with Ro60 170/4 apoptotic fibroblasts and anti-Ro60 was inhibited by  $45\% \pm 13\%$  in the presence of the TLR7 inhibitor, IRS661.

### **Conclusion:**

These data suggest that an alteration of the Ro60 domain which prevents Y RNA binding attenuates a permissive signal that is required for its participation as an antigen to form immune complexes in apoptotic cells and generate a TLR dependent proinflammatory cascade. Accordingly, the Y RNA moiety of the Ro/SSA ribonucleoprotein imparts a critical role in the pathogenicity of anti-Ro60 autoantibodies.

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