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The Unusual Cross-Reactivity of Anti Muscarinic Receptor 3 Monclonal Antibodies Derived from Salivary Glands of Sjögren's Syndrome Patients to Ro Peptides

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Background/Purpose: Sjogrens syndrome (SS) is a chronic autoimmune inflammatory disease the characterisitic features of which includes hypofunction of exocrine glands leading to decrease in salivation (dry mouth) and lacrimation (dry eyes). The presence of antibodies against Ro and La autoantigens serves as one of the hallmarks in the diagnosis of SS. Anti-muscarinic receptor 3 antibodies are another set of antibodies also found in SS patients. Muscarinic receptor 3(M3R) is a parasympathetic end organ seven transmembrane GPCR present on salivary and lacrimal glands, the stimulation of which is known to produce salivation and lacrimation. M3R has three extracellular domains (ECD) that plays an essential role in ligand binding and stimulation of the receptor. We found that monoclonal antibodies derived from salivary glands of SS patients that are positive for 2^{nd} and 3^{rd} ECDs of M3R are highly reactive to Ro peptides.

Methods: Monoclonal antibodies (MAbs) from salivary glands: Patients having dry eyes and dry mouth were classified according to AECG criteria into SS group and Do not meet criteria group (DNMC-Control). Plasmablasts (antibody secreting cells) with CD3- CD4- CD8- CD19+ CD27high CD38high IgG+ surface markers were sorted out using FACS from the salivary glands obtained following biopsy. The variable(V),diversity(D),joining(J), complementarity determining region(CDR) portions of heavy and light chain from each individual plasmablast were sequenced, amplified by PCR, cloned into a vector, transfected and expressed in HEK293A cell line. Ro and M3R Experiments: Reactivity of each Mab towards Ro were tested either by Bioplex 2200 or invitro transcription/translation system employing Ro52 and Ro60 labeled with ^{35}S-methionine and biotinylated-Lys or both methods. Reactivity to M3R peptides were determined by ELISA employing peptides encoding either 2nd (A.A. 213-218) or 3rd ECD (A.A.514-527) respectively.

Results: In the SS group, 23 Mabs were positive for Ro. 9 mabs were postive for 2nd ECD and 5

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were positive for 3rd ECD of M3R. 4 Mabs were positive for both the M3R domains. (Cutt off 2 S.D. above DNMCs average O.D.). We found that 7/9 (77%) of ECL2 +ve MAbs were positive for Ro whereas 4/5 (80%) of ECD3 +ve MAbs were positive for Ro. Four Mabs that were positive for both 2nd and 3rd ECD, all (100%) were positive for Ro. None of the Mabs in the DNMC group were positive for 2nd ECD.

Conclusion: We have found a sequence similarity between Ro and M3R ECDs. This high cross reactivity of anti-M3R antibodies with Ro possibly suggests a mechanism where antibodies formed against these shared portion of Ro with M3R could cross react with M3R and may cause inhibition of the receptor leading to major symptoms observed in SS. We are further studying functional aspects of anti-M3R antibodies on the receptor function and getting a significant higher inhibition from Mabs from SS when compared to DNMCs. Our future studies are also aimed at discovering the functional aspects of anti-M3R monoclonal antibodies on lacrimation and salivation when passively transferred to mice.

Disclosure: S. M. S. Quadri, None; **K. A. Koelsch**, None; **B. T. Kurien**, None; **V. M. Harris**, None; **R. H. Scofield**, None.

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