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Compartment-specific Gene Expression in Thymus and Thymoma associated with Myasthenia Gravis

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Background: The thymus is a crucial immune organ that plays a key role in the pathogenesis of various subtypes of the autoimmune disease myasthenia gravis (MG). The compartments (medulla and cortex) of the thymus act differently in terms of negative and positive T cells selection, normally generating both functionally relevant and self-tolerant T cells from immature hematopoietic progenitors. Thus, it is of importance to determine the differential expression in medulla and cortex of genes that are involved in these selection processes and potentially in thymus-dependent autoimmune diseases. Such studies have not been done in relation to thymoma-associated MG.

The major aim of the current study was to separate the medulla and cortex of human and mouse thymuses by laser microdissection microscope (LMD) and optimize this technique. Another important aim was to investigate the expression of autoimmunity-related genes (particularly muscle genes, including titin) in a potential model of thymoma-associated MG, i.e. in the largely muscle-deficient myogenin knock out mouse.

Material and Methods: Methods applied in this study in addition to LMD were quantitative (Taqman) RT-PCR and statistical analyses using Graph Pad Prism 6.0 software.

Results: The medulla and cortex were successfully separated by LMD. However, relatively valid results that were not biased by prolonged preparation times required that medullary and cortical regions were not successively microdissected in the same section but isolated from serial sections within the same time period after thawing and staining of each cryosection. Using this refined and highly standardized technique, the known (i.e. control) medullary genes AIRE, CD40 were reproducibly found to be expressed at high levels in the medulla, while key 'cortex-specific' genes like BETA5T, PRSS16, CD205, and CTSV were expressed at higher levels in the cortex than medulla, validating the applied method. The skeletal muscle genes titin and desmin were found to be over-expressed in the medulla compared to cortex in both adult human and wild type mouse thymus. In neonatal myogenin knock out mice (that are not viable) a separation of cortical and medullary regions was not achieved due to the tiny size of neonatal thymuses and their blurred cortico-medullary architecture. However, even in whole tissue extracts the expression of titin RNA was significantly reduced in neonatal myogenein knock out mice.

Conclusion: The finding that most skeletal muscle genes, including titin are preferentially expressed in the thymic medulla suggests that they are involved in the induction of tolerance towards muscle autoantigens. This helps to better understand the pathogenesis of MG. The finding that titin is significantly down regulated in myogenin knock mouse thymuses suggests a mouse model to elucidate the so far enigmatic molecular and cell biological basis of anti-titin autoimmunity in thymoma patients.