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Demonstration of the distribution of cholinergic nerve endings and nicotinic acetylcholine receptors by immunohistochemical and immunoflourescence techniques

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Objectives: The aim was to identify the distribution of α1,α7 nicotinic acetylcholine receptors(nAChRs) and Vesicular acetylcholine transporters(VAChT) which localizes cholinergic nerve endings in immune tissues of post-mortem humans by immunohistochemistry(IHC) and immunoflourescence(IF) techniques.

Methods: Human immune tissues were processed for Haemotoxyline & Eosin staining, IHC and IF. One set of tissue sections were labeled by primary antibodies, anti-VAChT and anti-α1,α7nAChRs and biotinylated anti-rabbit IgG for IHC. Second set was labeled with same primary antibodies and flouresceine(FITC) labelled anti-rabbit IgG as secondary antibody for IF. Immunofluorescence was observed by immunoflourescence microscope (Olympus,FSX100). Positive controls were skeletal muscle and cerebrum. The computerized images were analyzed depending on the intensity of immunostaining determined based upon a score of 0, 1+(focal staining, >10%), 2+(focal to diffuse staining, 10%>50%), 3+(diffuse staining, 50>100%).

Results: Immunohistochemical IR to α1nAChRs was identified in splenic capsule, red pulp and the subscapsular sinus, medullary cords & trabeculae of lymph nodes. Immunoreactivity of α7nAChR was expressed in macrophages predominant sites of spleen and lymph nodes. VAChTs was localized specifically where nAChRs was found. Peyer's patches and liver expressed low IR of anti- nAChRs, but absent anti-VAChTs. According to the IF results, trabeculae and subcasular sinuses of lymph nodes, the splenic capsule and red pulp expressed strong signals to anti-α1nAChR, but much less to α7nAChRs except the macrophages. Immunofluorescence expression of VAChTs was similar to IHC.

Conclusion: Immunohistochemistry and Immunoflourescence techniques confirm the distribution of cholinergic nerves in spleen and lymph nodes and their involvement in neuroimmune modulation through α1 and α7nAChRs.