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### **Immunohistochemical localization of Vesicular Acetylcholine Transporters and the Choline acetyl transferase enzyme activity in lymphoid tissues of Wistar rats and Balb/C mice**

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**Objective:** The aim was to localize the terminals of cholinergic nerves in immune tissues of Balb/C mice and Wistar rats using antibodies against vesicular acetylcholine transporter proteins (VACHT) in synaptic vesicles and choline acetyl transferase (ChAT) enzyme which synthesizes acetylcholine by immunohistochemical technique.

**Methods:** The murine immune tissues; spleen, lymph nodes, thymus, liver and Peyers' patches of small intestine were processed for Haematoxyline & Eosin staining and immunohistochemistry. The tissue sections were labeled by primary antibody, anti-VACHT and anti-ChAT and biotinylated anti-rabbit IgG. Labeled StreptAvidin Biotin method applied using Diaminobenzidene chromogen for light microscopic visualization. Skeletal muscle was used as positive control. 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:** Immunoreactivity (IR) to VACHT antibody was high (2+IR) in capsule and red pulp where reticular supporting framework was found but absent in white pulp of spleen in both murine species. The capsular & septal regions of thymus showed 2+IR while the capsule and subcapsular areas of lymph nodes showed 1+IR to VACHT. The IR to VACHT was absent in liver and Peyers' patches. IR to Anti-ChAT was similar to the distribution of VACHT in spleen, lymph nodes and thymus and was absent in Peyers' patches.

**Conclusions:** The presence of VACHT proteins and the ChAT enzyme activity in murine immune tissues confirms the neuroimmune modulation by cholinergic innervations distributed through the capsular and perivascular supporting framework of immune tissues.