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Anatomical evidence of cholinergic neuroimmune interactions in lymphoid tissues: an immunohistochemical study

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Although the immune system is known to be regulated by autonomic nerves, the cholinergic neuronal control is not well studied. The recent development of antibodies against the subtypes of muscarinic and nicotinic acetylcholine receptors (nAChR, mAChR), vesicular acetylcholine transporter protein (anti-VACHT) permits a greater reliability in the identification of cholinergic innervations. The cholinergic nerve endings and the distribution of α subunits of nAChRs in the lymphoid organs such as liver, spleen, Peyer's patches from terminal ileum and lymph nodes of post-mortem humans were studied by indirect immunohistochemistry. The tissues were processed for Hematoxylin and Eosin staining, were labelled by monoclonal anti-nAChR ($\alpha 1$, $\alpha 7$ subunits) and polyclonal VACHT antibody and linked to biotinylated anti-rat IgG. Labelled StreptAvidin Biotin technique was used; with DiaminoBenedene (DAB) as chromogen to detect the immunoreactivity by the development of brown colour. Skeletal muscle and cerebral cortex were used as positive controls. The intensity of staining was determined based upon a score of 0 - 3+ by qualitative computerized image analysis.

The $\alpha 1$ nAChRs were distributed in the capsule and red pulp areas of spleen and the subcapsular sinus, medullary cords and trabeculae of lymph nodes. A diffused (1+) distribution of $\alpha 1$ nAChR were observed in the hepatocytes and portal tract and a similar IR of $\alpha 1$ nAChR was observed in the germinal centres of Peyer's patches. In the liver, the IR of $\alpha 7$ nAChR was found to be low while the red pulp of the spleen showed an intermediate IR. In lymph node, the medullary cords and capsule expression was intermediately diffused. The IR to nAChRs observed in the macrophage predominant red pulp and trabeculae of spleen and lymph nodes and the absence in B lymphocytes was comparable with the findings of previous studies. The distribution of VACHT proteins localizes specifically at the sites where the nAChRs are found. This was observed in spleen and lymph nodes. The Peyer's patches expressed a low IR of anti-n AChRs, but in the liver, there was no IR towards anti-VACHTs. The findings showed that the neuronal parasympathetic cholinergic innervations of humans found in lymph nodes and spleen are possibly through nAChRs observed in T cell and macrophage abundant regions of these tissues.

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