Three-dimensional reconstruction of the pharyngeal tonsil innervation pattern in sheep

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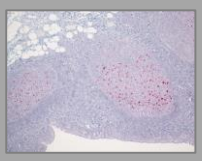
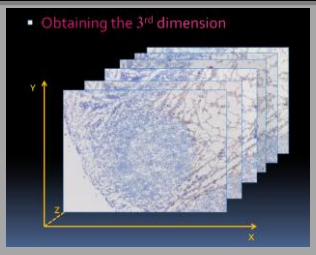
BACKGROUND

Scrapie is a neurodegenerative disease affecting sheep and goats, caused by an unconventional transmissible agent: the pathogen prion protein (PrPd). During a first silent phase of amplification inside lymph follicles, the pathogen reaches the peripheral nervous system and spread retrogradely to the central nervous system. If the most likely portal of entry in natural scrapie has been suggested to be the alimentary tract, other potential routes though to be effective experimentally. Sheep inoculated with scrapie intra-nasally have been showed to develop TSE. PrPd was essentially confined in the pharyngeal tonsils, making this lymph organ a possible portal of entry for the scrapie agent. In this context, we realised a three-dimensional reconstruction of the innervation pattern in the lymphoid compartments of the ovine pharyngeal tonsil.



PrPd revealed on ovine pharyngeal tonsillar paraffin sections after intranasal inoculation. PrPd was immunolabelled by a cocktail of two monoclonal antibodies: F89/160.1.5 and F99:97.6.1. Counterstaining with haematoxylin. The pathogen protein (red spots) was located in the germinal centre of some lymphoid follicles.

MATERIAL AND METHODS



Anmals Immunolabelling of pharyngeal_tonsillar FDCs with mAb: FBC-B1. Counterstaining with haematoxylin. MZ: mantle zone; LZ: light zone; DZ: dark zone. Bar = 200 µm

Obtaining the 3rd dimension

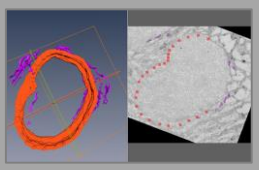
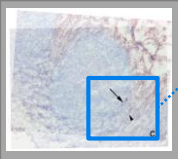
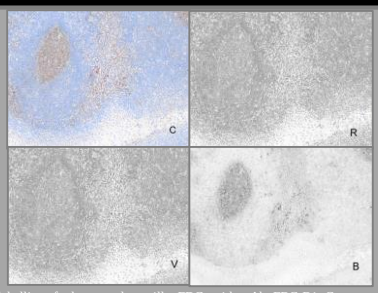


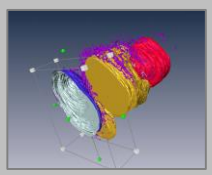
Image segmentation. In the right part of the screenshot, the whole pixels corresponding to the nerve fibers to reconstruct, were manually delimited (purple) and assigned to a file "fibers" common to all the images of the brick analyzed. The same operation was reproduced for the lymphoid follicles (red). In the left part of the screenshot, the correspondence and continuity of the delimited structures was verified and refined manually thanks to the visualization in 3D of the labeled pixels.



Images alignment: consecutive pictures were transformed in transparent images. The upper and lower images where manually aligned in the alignment module of Amira 4.0.1 software, thanks to the superposition of landmarks determined on consecutive images. Then, the nerve fibers continuity between two successive images (black arrows) were adjusted manually on the transparent pictures in original colors by zooming on areas of interest.

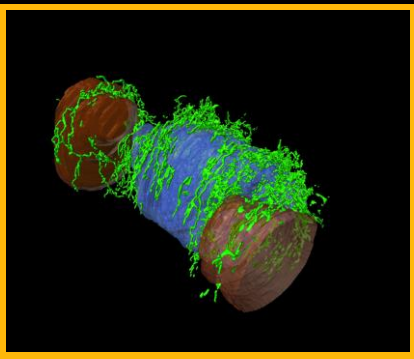


Immunolabelling of pharyngeal_tonsillar FDCs with mAb: FBC-B1. Counterstaining with haematoxylin. MZ: mantle zone; LZ: light zone; DZ: dark zone. Bar = 200 µm

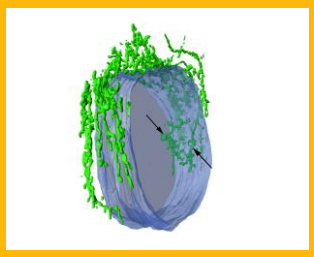


Transmission electron micrograph of an FDC cluster isolated from ovine pharyngeal tonsils. (*) FDCs, (L) lymphocytes. Bar = 1.5µm

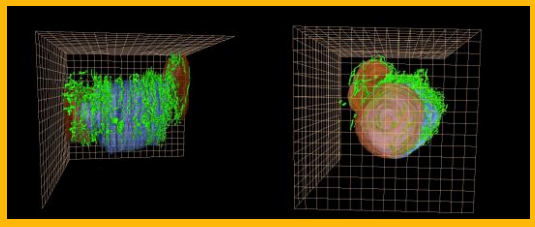
RESULTS



Example of 3D reconstruction of the innervation pattern of a lymphoid follicle chain inside an ovine pharyngeal tonsil. Only the central follicle (in blue) was entirely reconstructed. On both sides, stayed the extremity of the adjacent follicles (brown). GFAP+ nerve fibers (green), coming from the tonsil's conjunctive axis, walked along the follicle in direction of the respiratory epithelium. In this way, their density diminished.



Inside the entire reconstruction, only two nerve fibre extensions **(arrows)** invaded one of the lymphoid follicles.



Measure of the size of the reconstructed lymphoid follicle (blue). Dimensions of the central follicle: height = 800 µm, width = 500 µm, length = 1400 µm. Scale grid : 100 µm.

CONCLUSIONS

The computing 3D reconstruction ensures a representation closer to the reality than an analysis on histological slides and allowed to evaluate the frequency and distribution of the nerve fibres surrounding lymphoid follicles of the pharyngeal tonsil. Because some nerve fibres were detected inside the lymphoid follicles, neuro-immune connections between nerve endings and immune cells responsible for prions amplification could be one of the link between lympho- and neuro-invasion.