Study of the cellular prion protein expression by the follicular dendritic cells in ovine pharyngeal tonsils.

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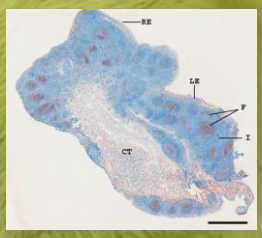
BACKGROUND

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 and hamsters 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.

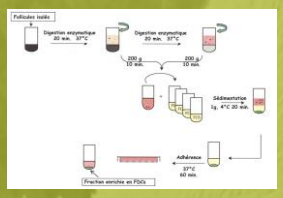


Immunolabelling of ovine pharyngeal tonsillar cryosections with mAb: FDC-B1. Counterstaining with haematoxylin. The deeply folded mucous membrane is covered by a respiratory epithelium (RE) invaded in some locations by lymphoid cells providing a route to a reticular lymphoepithelium (LE). Inside the folds, 1 or 2 layers of secondary lymphoid follicles (F) are aligned side by side. The interfollicular area (I) is highly infiltrated by lymphoid cells. The central axis of the tonsil is filled with a loose collagenous connective tissue (CT) rich in blood and lymphatic vessels. Bar = 2mm

OBJECTIVE

Our study analysed the possible implication of the follicular dendritic cells (FDCs) network in the replication of the scrapie agent in the nasopharyngeal ovine mucosa.

MATERIAL AND METHODS

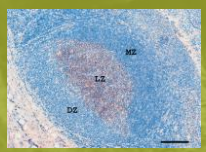


Isolation of FDC clusters (Thielen *et al*., 2002)

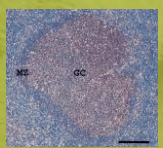
Pharyngeal tonsils were obtained from clinically healthy sheep and were cut in 1mm thick slices. The lymphoid follicles were isolated from the connective and epithelial tissues, submitted to enzymatic digestions and sedimentation gradient. Cellular prion protein expression was analysed on FDC clusters by immunoperoxidase and immunogold labelling after incubation with moAb Pri909.

RESULTS

The immunoreaction of the FDCs was limited to the light zone of the germinal centre of the lymphoid follicles. Some extensions were able to run through the dark zone and the mantle zone. Immunolabelled PrPc was detected in both the light and the dark zones of the germinal centre. The mantle zone remained unstained.

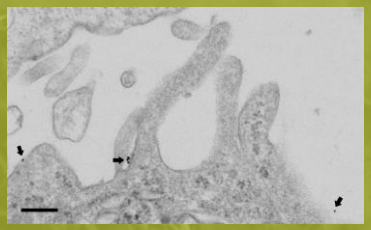


Immunolabelling of pharyngeal tonsillar FDCs with mAb: FBC-B1. Counterstaining with haematoxylin. MZ: mantle zone; LZ: light zone; DZ: dark zone. Bar = 200 µm



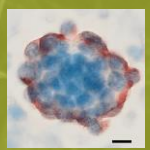
Immunolabelling of a secondary lymphoid follicle with mAb: Pri 909. Counterstaining with haematoxylin. MZ: mantle zone; GC: germinal centre. Bar = 200 µm

The precise localisation of Pri 909 moAb labelling has been confirmed on ultrathin sections after immunogold analysis by electron microscopy. At high magnification, gold particles were localised on the extensions of the FDC cytoplasmic membrane.

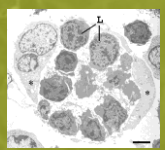


Immunogold labelling with mAb: Pri 909 of an FDC cluster isolated from ovine pharyngeal tonsils. Gold particles (arrowheads) line up along the cell surface of FDC cytoplasmic extensions. Bar = 200 nm

The FDC membrane engulfed 8 to 22 compacted lymphocytes and one or two FDC nuclei were distinct. After immunoperoxidase labelling, PrPc was located on the membrane extensions wrapped around lymphoid cells.



Immunolabelling of an FDC cluster isolated from ovine pharyngeal tonsils with mAb: Pri 909. Counterstaining with haematoxylin. Bar = 10 µm



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

CONCLUSION

Our results demonstrate that the FDC of the ovine pharyngeal tonsils express PrPc on their cytoplasmic membrane in the same way of the FDC resident in lymphoid organs known to be sites of replications of PrPd during a scrapie infection. This support the hypothesis that the pharyngeal tonsils may be involved in scrapie pathogenesis.