Analysis of key and dynamic events of host-pathogen interactions by immuno-labelling and Correlative Light Electron Microscopy. Easy and fast approaches.

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Electron microscopy (EM) plays a key role in the identification and characterisation of cellular compartments, pathogens and host - pathogen interactions. Transmission and Scanning Electron Microscopy (TEM and SEM) provide 2D and 3D morphological analyses with optional detection and localisation of specific antigens by immuno-gold-labelling. Correlative Light and Electron Microscopy (CLEM) which provides a correlation between the light microscopy (LM) and EM signal is mandatory if key, rare and/or dynamic events of host-pathogen interactions need to be studied at high-resolution.

Immuno-gold-labelling and CLEM are usually not simple techniques and require the development and adaptation of protocols depending on the questions asked.

Through the following projects and by using easy and fast approaches in immuno-labelling and CLEM, we want to analyse by TEM and SEM the localisation of specific proteins in infected cells.

Human Cytomegalovirus virus (HCMV) is one of the principle causative agents of transplant rejections globally and is also prevalent as a pathogen in pregnant women where it is linked to birth defects. In the ARPE-19 cell line infected with HCMV, we use a fully human antibody (derived from a recovered patient) to target the HCMV UL44 proteins. Based on flow cytometry and confocal microscopy results, we observed that UL44 is located on the cell surface which was not expected because UL44 is known to be a mostly nuclear protein. It has never been reported to be on the surface of infected cells. Using a secondary antibody double-labelled with both fluorescent and gold probes together with the "Correscopy kit" for CL-SEM, we can confirm by labelling for both fluorescence and electron microscopic observation with a single immunostaining and classic EM procedure, the localisation of UL44 on the cell surface.

Enterovirus 71 (EV71) causing Hand, Foot and Mouth Disease, is regarded as the most important neurotropic virus worldwide. In the Motor Neuron cell line NSC-34 infected with EV71, we try to visualise the expression of peripherin and investigate whether peripherin co-localises with EV71. Using secondary antibodies double-labelled with fluorescent and gold probes and the FEI Tecnai with Icorr electron microscope, we can analyse by both fluorescence and electron microscopic observation with a single immunostaining and classic EM procedure, the localisation of peripherin and EV71 in the same cells.

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