Correlative light-electron microscopy investigations of single-cell dynamics in Mycobacteria tuberculosis infected human macrophages

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Virulent Mycobacteria tuberculosis (Mtb) infection can cause pyroptosis. This programmed necrotic form of cell death can proceed activation of inflammasomes, which are cytosolic sensors of infection. We apply live cell fluorescence imaging techniques along with focused ion beam scanning electron microscopy (FIB-SEM), to obtain correlative light electron microscopy (CLEM), in order to investigate single cells over time and reveal the dynamics of infection. Cells cultivated on polymer microscope coverslips, with custom-made correlation patterns, allow precise relocation of single cells when proceeding from light microscopy to electron microscopy. Targeted 3D FIB-SEM provide ultrastructural data on interesting events observed during live cell fluorescence microscopy. We have found that virulent Mtb can trigger phagosome rupture in THP-1 macrophages, which recruits host membranes to the escaped bacteria. Furthermore, activation of the canonical NLRP3 inflammasome, pyroptosis and release of IL-1b can follow in cases of failed containment. 3D FIB-SEM at time-points distinguished by the recruitment of different fluorescent reporters to the vicinity of Mtb bacteria exposed distinct ultrastructural changes in human THP-1 macrophages. By further developing this CLEM platform we envision new temporal and ultrastructural insights into the complex and dynamic processes of Mtb infections in single cells.