MULTIMODAL MICROSCOPY TO STUDY THE FATE OF BACTERIOPHAGES IN LIVER SINUSOIDAL ENDOTHELIAL CELLS

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ABSTRACT:

Blood from the gastrointestinal tract reaches the liver via the portal vein, bringing along a variety of microbial products. Thus, the liver faces continuous exposure to many pathogens. Yet, infection occurs only rarely owing to the powerful innate and adaptive immune responses of the liver. For decades, the uptake of virus has been studied in connection with infection, but very few studies were designed to look into clearance mechanisms, and typically focused on Kupffer cells. Liver sinusoidal endothelial cells (LSECs), however, are critical in pathogen detection, capture and antigen presentation, and have a remarkable ability remove these complexes either through internalization and degradation via receptor-mediated endocytosis [1, 2], or by passing them through 50-200 nm transmembrane pores, called fenestrations, for processing by the surrounding hepatocytes [2]. Because of the size of these pores, their presence (and thus proper functioning of the cells [3]) can only be confirmed with super-resolution techniques, and at present the dynamics have only been captured using structured illumination microscopy (SIM). However, SIM requires a high photon load and is highly susceptible to photobleaching, thus it can be difficult to capture the dynamics of small (and sparsely-labeled) targets. In contrast to SIM, deconvolution (DV) microscopy requires a much lower photon load and is well-suited to long-term time-lapse imaging of dim samples, but the resolution is insufficient to visualize fenestrations. To combat these challenges, we first used time-lapse DV microscopy to follow the interaction between LSECs and GFP-labeled bacteriophages, and afterwards confirmed the presence of fenestrations in the same cells using SIM. By combining these imaging modalities with quantitative uptake studies, we were able to track the fate of bacteriophages from the time of their incubation with LSECs through endocytosis, and to eventual degradation in lysosomal compartments. This multimodal approach thus provides greater insight into both the mechanisms and timing underlying viral clearance by the liver.

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