INVESTIGATION OF BKPYV LIFE CYCLE, ACTIVATION OF INNATE IMMUNE RESPONSES AND MEMBRANE REMODELING IN MICROVASCULAR ENDOTHELIAL CELLS

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Polyomaviruses (PyVs) are small double-stranded DNA viruses that occur widely in nature. Among the 14 known human PyVs, three are particularly relevant: BK Polyomavirus (BKPyV), JC polyomavirus, and Merkel cell carcinoma Polyomavirus. BKPyV, with a global prevalence of approximately 80% in adults, typically causes asymptomatic primary infection. After initial infection, it disseminates via the bloodstream and establishes persistence in the urinary tract. Reactivation of BKPyV can occur in immunocompromised patients, leading to complications such as nephritis or graft loss[1-2]. Unfortunately, there are currently no specific antiviral treatments available for BKPyV infection.

Recently, for the first time, human microvascular endothelial cells from the bladder (HMVECs bd) have been proposed as viral reservoir cells due to their unique response to infection, involving interferon (IFN) production[1]. In this study, we aim to unravel the molecular details of BKPyV replication and the underlying activation of the IFN response in primary HMVECs bd.

We found that at early times post-infection BKPyV virions are located inside internalized monopinocytic vesicles and later can be detected in late endosomes, lysosomes, tubuloreticular structures, ER and vacuoles-like vesicles. Interestingly, we also noted abundant lipid droplets and remodeling of ER membranes, suggesting a viral-induced ER stress response.

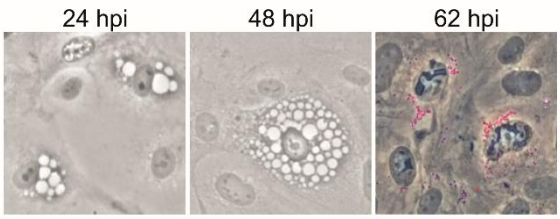
We detected that the production of virus progeny starts at 36hpi while increased permeability of the cell membranes and peaks of virion release coincide with leakage of viral and cellular DNA to cytosol around 60hpi.

Leaked viral and cellular DNA colocalize with and activate cGAS leading to activation of STING and consequent transcription of IFN β and IFN-related genes, ISG56 and CXCL10. Importantly, the IFN response to BKPyV primarily results from IRF3 activation, not NF-kB. Overall, the IFN response is moderated compared to other stimuli.

The reduction of the IFN response by the cGAS inhibitor, G140 highlights the importance of the cGAS-STING pathway in the innate immune response of HVMECS bd to BKPyV.

Although the BKPyV life cycle in HBMVECs follows a similar pattern and kinetics to that of renal proximal endothelial cells (the primary target cells for the virus during reinfection), the formation of tubuloreticular structures, ER membrane remodeling, vacuole-like vesicles, and lipid droplets—presented here—are not well-documented yet as a feature of BKPyV infection.

Concerning the innate immune responses, our results here suggest that moderate activation of the innate immune response via the cGAS-STING pathway at a late stage of infection is a unique virus strategy that supports viral persistence. This approach ensures that some virus is produced, while simultaneously triggering an antiviral state in cells.



Formation of vacuoles like-vesicles and lipid droplets during BPyV infection of HMVECs bd. Cell morphology was assessed by bright-field (BF) microscopy at 24, 48, and 62 hpi. At 62hpi a stain for detection of neutral lipids (red) was used.

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