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EXPLORING THE MOLECULAR MECHANISMS USED BY HUMAN BK POLYOMAVIRUS TO ACTIVATE IMMUNE RESPONSES IN "RESERVOIR CELLS"

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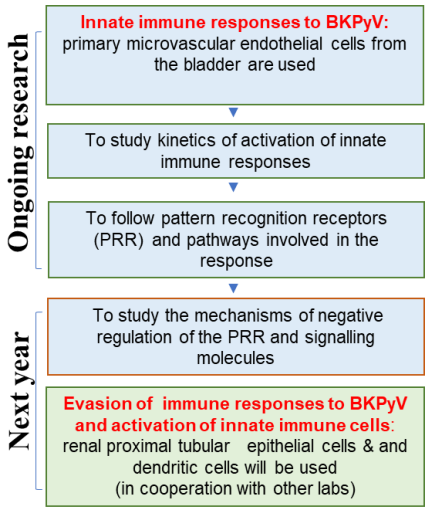
At present, the Polyomaviridae family contains 14 human polyomaviruses (PyVs), among the most important are Merkel cell polyomavirus (MCPyV), the etiological agent of 80% of Merkel cell carcinomas, JCPyV and BKPyV causing progressive multifocal leukoencephalopathy and nephropathy, respectively, in immunocompromised individuals. Primary infection by polyomaviruses occurs in early childhood and is usually asymptomatic.[1,2] After initial primary infection and dissemination, PVs persist in the organism by a not well-known mechanism. In fact, it has been suggested that innate immune responses in the reservoir cells may contribute to PyV persistence.[3,4] Although two cellular models have emerged recently to study the mechanism of BKPyV infection, there are still many gaps in the understanding of these models. One of the models is human microvascular endothelial cells (HMEVC) from the bladder (bd) or lung, which respond to BKPyV by producing interferon (IFN), and the other is renal proximal tubular epithelial cells (RPTEC), which do not respond immunologically to the virus. Cells that respond to BKPyV with production of IFN have been postulated as reservoirs of the BKPyV. In our group, a part of our research focuses on understanding the mechanisms of innate immune response activation and modulation in response to BKPyV infection.

Since our previous studies showed that mouse polyomavirus (MPyV) induces a moderate IFN response in host cells as a results of DNA sensing via cGAS-STING pathway[5], we hypothesized that: i) in the 'reservoir cells' (HMVECs bd) BKPyV activates cGAS-STING pathway to produce IFN responses that lead to lower levels in viral progeny, thereby contributing to viral persistency and that ii) cGAS-STING pathway is heavily regulated either by post-translational modifications of cGAS and/or STING and/or possibly by interactions between viral proteins and proteins of the innate immune system.

To follow our hypotheses, we first investigated the life cycle of BKPyV in primary HMVECs bd during 96 hours post infection (hpi). We have found that the viral early antigen LT can be detected after 12hpi, while the production of the late antigen VP1 and massive replication of viral DNA started between 24–36hpi. Low levels of virion release occur from 48hpi (whether or not these virions are new progeny needs to be determined) with a marked increase at 60hpi. Signs of cell toxicity are apparent at 72hpi. Additional experiments to better understand the BKPyV life cycle in HMVECs bd will be carried out.

Next, we had shown that HMVECs bd launch an IFN response at late times, around 72hpi. The response is characterized by upregulation of IFNB, ISG56, CXCL10, and CLCL20. Further, we followed the possible involvement of cGAS sensor by observing mutual colocalization of viral DNA (stained by FISH) and cGAS in the cytosol and found that at early times post-infection (24h), cGAS colocalized with spot pattern clusters of viral DNAs from the incoming virus. Later, at 62hpi, we also found cGAS colocalizing with viral DNA leaked from the nucleus into the cytosol. We suggest that the clustering of cGAS with incoming viral DNA corresponds to sorting the virus to autophagosomes. In fact, colocalization of viral particles and LC3B, the central protein in autophagy, was detected at 24hpi. Further studies will be carried out to understand the role of cGAS mediated autophagy in BKPyV infection. On the other hand, the colocalization of cGAS with viral DNA at 62hpi very likely results in the activation of cGAS. In agreement, activation of STING was detected at high levels at 62 and 72hpi. Ongoing experiments are being carried out to confirm the cGAS activation by determining the levels of cGAMP.

Finally, we have prepared cell lines expressing tagged STING and cGAS proteins, plasmids coding for the tagged proteins, and viral mutants to study the regulation of the IFN responses in RPTE and HMVECs bd. In the table below, a summary of our research is presented.



Acknowledgement
This study was supported by „The project National Institute of virology and bacteriology (Program EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union – Next Generation EU.

REFERENCES

1. Knowles W. A.: Adv. Exp. Med. Biol. *577*, 19 (2006).
2. Kamminga S, et al.: PLoS One *13*, e0206273 (2018).
3. Popik W., et al.: Viruses *11* (2019).
4. An P., et al.: PLoS Pathog *15*, e1007505 (2019).
5. Ryabchenko B., et al.: FEBS J. *288*, 5964 (2021).