EBV LMP-1 FORMS A K^+ SELECTIVE ONCOCHANNEL THAT SUPPORTS LYTIC VIRUS RELEASE

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Viruses manipulate ion membrane potentials to facilitate their replication, pathogenicity, and persistence. Indeed, many viruses encode ion channels (known as viroporins) to spark catalytic activities, induce membrane deformation, and facilitate virus invasion, uncoating, trafficking, or release. With K^+ efflux a known trigger of inflammasome activation, and the inflammasome recently linked to EBV lytic activation, we asked if EBV regulates plasma membrane K^+ potential during its lytic phase. Using a flow cytometric assay to measure K^+/Tl^+ transport, we find that K^+ transport increases as EBV progresses through its lytic phase. Blocking K^+ efflux further prevents virus production and release without inhibiting viral DNA replication. A search for hidden Markov model similarities to known K^+ channels revealed similarity between the last transmembrane span of EBV Latent membrane protein-1 (LMP-1) and the HIV-1 viroporin Vpu.

LMP-1 is known to constitutively activate signal transduction pathways via elements in its cytoplasmic tail in a manner that mimics the B cell co-stimulatory molecule, CD40. LMP-1 is also considered to be the most potent EBV oncoprotein. Despite detailed characterization during latency, little is known about LMP-1's intrinsic properties or if/how it contributes to EBV lytic replication. Employing EBV^+ Burkitt lymphoma cells in latency I (lacking LMP-1 expression), we find that lytic induction leads to full length LMP-1 expression and its progressive localization to the plasma membrane over the course of the lytic phase. De novo modeling predicts a circular arrangement of LMP-1's last three transmembrane spans, and electrophysiology of epithelial cells expressing LMP-1 reveals a high flicker type K^+ channel activity at negative voltages with a single-channel conductance of 105 +/-8 pS. To determine if the LMP-1 induced K^+ channel function is intrinsic to LMP-1 or indirect (via signaling), we removed all of the known cell signaling components leaving only the transmembrane spans intact. This significantly altered the biophysical properties of the channel, opening the channel at negative *and* positive potentials as well as increasing conductance to 250 pS, indicating an intrinsic channel formed by the transmembrane spans. Given these findings, we believe LMP-1 to be a small-conductance K^+ channel resembling an inward rectifying, Kir-type or SK-type channel. The LMP-1 channel likely promotes virus release during the lytic phase and may further have critical functions in latency related to B cell transformation or tumorigenesis. To our knowledge, LMP-1 represents the first viral oncochannel – and a novel target for channel-blocking antiviral/anticancer agents.