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Transient induction of lytic Kaposi's sarcoma-associated herpesvirus (KSHV) genes in KSHV-infected endothelial cells exposed to hypoxia

P Velasco*, L Dutcher, DA Davis and R Yarchoan

Address: HIV and AIDS Malignancy Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

* Corresponding author

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Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus-8 (HHV-8) is the causative agent of Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and a form of multicentric Castleman's disease (MCD). Two of these tumors, KS and PEL, tend to arise in areas of the body that are relatively hypoxic. Exploring this phenomenon, our group has previously found that KSHV in PEL tumor cell lines is activated to lytic replication by hypoxia and that certain KSHV genes are activated by hypoxia inducible factors (HIF-1 and HIF-2) through functional hypoxia response elements (HRE) (Davis et al., *Blood*, 2001, 15, 3244–50; Haque et al., *J. Virol.*, 2006, 80, 7037–51). KS tumor cells are thought to be derived from endothelial cells or their precursors, and in the current study, we have addressed the question of whether KSHV was similarly activated to lytic replication in KSHV-infected endothelial cells.

To investigate this, we infected primary dermal microvascular endothelial cells (DMEC) derived from human foreskin with a recombinant KSHV (rKSHV.219) that was engineered to contain red fluorescent protein (RFP) controlled by the lytic PAN RNA promoter as a lytic marker and green fluorescent protein under the control of human elongation factor 1-α promoter as a latent marker (gift of Dr. Jeff Vieira). Short-term (<24 hours) exposure of these KSHV-infected DMEC to hypoxia (1% O_2) resulted in an initial increase in the expression of KSHV lytic genes (including RTA and viral interleukin [IL]-6) and secretion of KSHV into the supernatant. However, when the infected DMEC were cultured in hypoxia for > 24 hours, there was a subsequent downregulation of KSHV lytic genes and of the production of virus despite continued upregulation of HIF-2 in the cells. We hypothesized that this late downregulation might be from production of one or more cellular factors with KSHV-suppressive activity in these cells upon exposure to hypoxia. We focused on IL-1β, IL-6, IL-8, tumor necrosis factor alpha (TNFα) and vascular endothelial growth factor (VEGF), as these are known to be produced by hypoxic endothelial cells and to be expressed by KSHV-infected cells *in vitro* and in KS lesions. IL-6, IL-1β and VEGF had no effect on lytic replication and IL8 increased replication in the KSHV-infected DMEC. However, TNFα blocked the expression of RTA, vIL6, ORF45 and K5 proteins and suppressed production of KSHV virions in the supernatant. Furthermore, when the cells were treated with blocking antibodies directed against human TNFα, KSHV lytic activation in the hypoxic DMEC was partially restored. Interestingly, established monolayers of KSHV-infected DMEC cells cultured in hypoxia out-survived normoxic cells cultured in parallel; this provided evidence that suppression of KSHV by TNFα was not simply from cell killing. These results suggest that production of TNFα by KSHV-infected endothelial cells may limit activation of lytic KSHV replication by hypoxia and that this effect may promote survival of KSHV-infected cells under hypoxic conditions and contribute to

the pathogenesis of KS. This research was supported in part by the Intramural Research Program of the NIH, NCI.