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Molecular Virology of KSHV in the Lymphocyte Compartment—Insights From Patient Samples and De Novo Infection Models

Farizeh Aalam and Jennifer Totonchy*

Biomedical and Pharmaceutical Sciences, Chapman University School of Pharmacy, Irvine, CA, United States

The incidence of Kaposi’s sarcoma-associated herpesvirus (KSHV)-associated Kaposi Sarcoma has declined precipitously in the present era of effective HIV treatment. However, KSHV-associated lymphoproliferative disorders although rare, have not seen a similar decline. Lymphoma is now a leading cause of death in people living with HIV (PLWH), indicating that the immune reconstitution provided by antiretroviral therapy is not sufficient to fully correct the lymphomagenic immune dysregulation perpetrated by HIV infection. As such, novel insights into the mechanisms of KSHV-mediated pathogenesis in the immune compartment are urgently needed in order to develop novel therapeutics aimed at prevention and treatment of KSHV-associated lymphoproliferations. In this review, we will discuss our current understanding of KSHV molecular virology in the lymphocyte compartment, concentrating on studies which explore mechanisms unique to infection in B lymphocytes.

Keywords: KSHV, HHV8 (KSHV), virus-host interaction, B lymphocytes, immune evasion, hematological malignancies

INTRODUCTION

KSHV (HHV8) belongs to the gamma-herpesvirus family and is associated with both lymphoid and non-lymphoid cell tumors in humans (Chang et al., 1994). KSHV-associated malignancies occur primarily in the context of immunodeficiency. KSHV is the etiologic agent of Kaposi’s sarcoma, as well as the B cell lymphoproliferative disorders, primary effusion lymphoma (PEL), and multicentric castleman disease (MCD) (Chang et al., 1994; Cesaran et al., 1995) as well as the recently discovered KSHV inflammatory cytokine syndrome (KICS) (Uldrick et al., 2010).

Despite nearly three decades of research, not much is known regarding the early stages of development for KSHV lymphoproliferative disorders and the person-to-person transmission of KSHV. This can be partly explained by the host range limitation and broad in vitro cellular tropism of KSHV (Blackbourn et al., 2000; Bechtel et al., 2003). During the latent phase of infection, viral gene expression is limited and KSHV is maintained as an extrachromosomal episome; persisting for the lifetime of the individual (Ueda, 2018). Like other herpesviruses, KSHV can become lytic under some physiological conditions (Grundhoff and Ganem, 2004; Li et al., 2014; Johnston et al., 2019; Wei et al., 2019). The process by which the lytic switch occurs and the relative contributions of...
lytic/latent phases to KSHV persistence are poorly understood. This is partly because expression and activity of the KSHV regulatory proteins appear to be cell type and tissue-specific and in vivo niches for persistence in humans remain poorly characterized (Rivas et al., 2001; Koch et al., 2019). There are significant gaps in our understanding of how KSHV targets B cells for infection and how the virus manipulates B cell physiology in the development of PEL and MCD (Figure 1). Further study of KSHV molecular virology in the lymphocyte compartment is needed to understand the pathogenesis of KSHV-associated lymphoproliferation so that effective treatment paradigms can be developed. In this review, we explore our current understanding of KSHV biology in B cells concentrating on studies which use de novo infection of human B cells, analysis of patient samples from KSHV lymphoproliferative disease, and relevant lymphoma cell lines. We have intentionally omitted discussion of KSHV manipulation of cytokine expression and signaling from this work as it is complex, and we have recently reviewed the topic comprehensively elsewhere (Alomari and Totonchy, 2020). Moreover, we have omitted discussion of humanized mouse models for KSHV infection as they have also been reviewed very recently (Münz, 2020).

**B LYMPHOCYTE-SPECIFIC MOLECULAR VIROLOGY OF KAPOSI’S SARCOMA-ASSOCIATED HERPESVIRUS**

**Entry Into B Cells**

HHV-8 DNA is detectable in the B cells from both HIV+ and HIV-PEL and MCD cases (Dupin et al., 1999). Interestingly, KSHV isolated from EBV+PEL cells is able to infect B cells from seronegative patients (Mesri et al., 1996). Phylogenetic analysis and the association of KSHV infection with pathological lymphoproliferations are sufficient to characterize KSHV as a lymphotropic gamma-herpesvirus. However, primary B cells and B lymphoma cell lines show poor susceptibility to KSHV infection in vitro compared to adherent cell lines (Buchtel et al., 2003). The extensive in vitro susceptibility of adherent cell lines can partly be explained by the presence of various cellular receptors used by the viral glycoproteins for attachment and entry (Akula et al., 2001a; Akula et al., 2001b; Akula et al., 2002; Rappocciolo et al., 2008; Hahn et al., 2009; Chen et al., 2019; Großkopf et al., 2019; Muniraju et al., 2019).

KSHV virion attachment to adherent cells can be facilitated through heparan sulfate proteoglycans on the host cell surface.
dependent manner (Hensler et al., 2014), but whether this KSHV fusion protein, binds to DC-SIGN

Finally, the KSHV glycoprotein gB, which is presumed to be the KSHV entry receptor for B cells has not been formally studied. xCT, the light chain has been shown to be involved in KSHV fusion and entry in several cell lines. Although, its mRNA expression is undetectable in CD19+ PBMCs (Kaleeba and Berger, 2006a; Kaleeba and Berger, 2006b) xCT is highly expressed on the surface of PEL cell lines and targeting it by xCT selective inhibitor, induces apoptosis in caspase dependent manner. Selective inhibition of xCT in immune deficient mouse xenograft model proves that it plays key role in tumor progression, survival, and growth of PEL cells (Dai et al., 2014). The expression of xCT can be induced by KSHV miRNAs conferring permissiveness to KSHV in murine microphages and HUVEC cells. Additionally, the expression of xCT within the KS lesion is correlated with the tumor stage (Qin et al., 2010).Whether KSHV miRNAs and change in redox balance contribute to upregulation of xCT in primary B cells to increase the KSHV permissiveness, remains to be answered.

To date, no comprehensive studies been done on primary human B cells samples to elucidate the cellular receptors involved in KSHV entry into B lymphocytes or the individual and collective contributions of KSHV glycoproteins to this process. Further studies are needed to determine these important interactions to facilitate the rational design of vaccine strategies that will effectively limit the establishment of infection in the lymphocyte compartment.

**Manipulation of the Cell Cycle**

KSHV can establish latent infection in many adherent cell lines, including human and non-human cells of epithelial, endothelial, and mesenchymal origin (Bechtel et al., 2003). Previous studies in primary human B cells report that infection is lytic, particularly in the absence of T cells, but what controls the lytic switch in these cells remains to be established (Myoung and Ganem, 2011a). In addition to T cell control of latency, B cell immunophenotype and activation state have been implicated as factors influencing the lytic/latent balance in B cells (Rappocciolo et al., 2008; Hassman et al., 2011; Myoung and Ganem, 2011a), as well as the immunological status of the individual and the presence of other pathogens (Gregory et al., 2009).

Although the latent phase of infection allows viral persistence and immune-evasion, the production of viral progeny and viral transmission and spread between the cells, depends on the lytic phase. *De novo* infected PBMCs exhibit simultaneous expression of numerous latent and lytic markers at the very beginning of the infection (Purushothaman et al., 2015). This short lytic replication seems to be a prerequisite for the establishment of the latent phase in PBMCs infected with EBV (Halder et al., 2009). Nevertheless, the lytic gene expression is not required for KSHV infection of PBMCs before or after EBV infection or mitogenic activation (Faure et al., 2019). Do B cells represent a significant source of KSHV virions during human infection? The early lytic gene K8 (K-bZIP), a cell cycle regulator showing homology to EBV’ Zta, is required for viral lytic DNA replication and virion production in PEL cell lines (Wu et al., 2002; Lefort and Flamand, 2009). Its expression concurs with augmented C/EBPζ, p21 and p27 in the nucleus, causing the cell arrest in G1
Lymphoproliferative diseases. This prolonged G1 arrest is as a result of K8 binding to CKD2, interfering its kinase activity, giving ample time for viral early gene transcription and translation (Izumiya et al., 2003a). K8 also interacts with p53 inhibiting its transcription, preventing apoptosis (Park et al., 2000). However, in another study by Hollingworth et al., lytic replication in PEL cells was shown to require S phase entry (Hollingworth et al., 2020). Replication and transcription activator (RTA) is a protein encoded by ORF8 has been shown to co-localize with K8 within the nucleus of the PEL cells, and its association with the K8 (Izumiya et al., 2003b) can initiate lytic reactivation from latency by binding to a particular sequence on the host and viral DNA further modulating the transcription of viral and host regulatory genes throughout KSHV lytic reactivation (Kaul et al., 2019). Viral DNA replication is controlled by both transcriptional coactivator p300 and CBP. P300 was shown to be involved in the oncogenesis of PEL by driving B cell proliferation and inhibiting KSHV lytic replication. Knockout of p300 in PEL cells decreased KSHV genome copy number and virion production by suppressing lytic gene expression, possibly maintaining the latency of KSHV via binding with ATF3 (Sun et al., 2020). Nonsense-mediated mRNA decay (NMD) is an RNA quality control implemented by the cells to restrict the action of the RNA viruses and serve cellular quality control. Interestingly, viral RTA mRNA is targeted by NMD, impeding KSHV lytic reactivation in PEL cells (Zhao et al., 2020). However, KSHV has evolved to overcome some of these quality controls and exonuclease activities by circularizing its structural and regulatory RNAs incorporated into the virions (Abere et al., 2020). Taken together, the current literature demonstrates that multiple layers of both viral and cellular regulation influence KSHV latency and lytic reactivation in B cells. It is notable that most of this work has been done in PEL cells, and future studies investigating how KSHV manipulates the cell cycle and cell type-specific control of latency and reactivation in primary B lymphocytes will be critical for understanding early events in KSHV infection and pathogenesis of KSHV-associated lymphoproliferative diseases.

Kaposi’s Sarcoma-Associated Herpesvirus Immune Evasion

KSHV infection persists for the lifetime of the host and, like all herpesviruses, KSHV must have an arsenal of mechanisms for evading host immunity in order to accomplish this. Lymphotropic gamma-herpesviruses are particularly interesting in this regard because they can manipulate and evade the host immune system via mechanisms that require direct infection of immune cells. Moreover, the inflammatory nature of KSHV-associated malignancies indicates that KSHV immune-evasion mechanisms may also directly contribute to pathogenesis in KSHV-associated diseases. Indeed, this immune-evasion is manifested at the transcriptional level within the first few hours of infection, by hampering the expression of immune response genes and inducing the proapoptotic regulators in BJAB cells (Naranatt et al., 2004). KSHV can infect both B and T cells in tonsil primary cell culture, however evidence suggests that infection of T cells is abortive (Myoung and Ganem, 2011b). Moreover, there is reciprocal activation of T cells by KSHV-infected B cells and contact-dependent control of KSHV lytic reactivation by T cells in ex vivo tonsil cultures, and in this system the activation of T cells is independent of both KSHV antigen and MHC restriction (Myoung and Ganem, 2011a).

Activated, KSHV infected B lymphocytes from PBMC and tonsils show downregulation of MHC class I (HLA-A, HLA-B, and HLA-C) within 24 h of infection as well as decreased expression of CD20 (Rappocciolo et al., 2008). Modulation of MHC class I expression is also observed in PEL derived B cell lines and is thought to be partially due to reduced expression of the TAP-1 gene. Importantly, this MHC-I modulation can disrupt cytotoxic T lymphocyte surveillance of KSHV infected cells (Brandr et al., 2000), aiding in KSHV persistence and tumorogenesis in B cells. The CD20 low phenotype of KSHV infected cells is also present in MCD and may limit B cell-targeted treatment options for MCD patients. However, these patients still show clinical benefit from rituximab (an anti-CD20 monoclonal antibody) treatment (Naresh et al., 2009).

KSHV encodes four viral interferon regulatory factors (vIRFs). These proteins have minimal homology to human IRFs, but vIRF1, vIRF2, and vIRF3 are known to bind DNA elements similar to their human IRF counterparts (Lubyova and Pitha, 2000; Park et al., 2007; Hu et al., 2016). vIRFs exert their regulatory role at varying levels ranging from hampering the antiviral interferon response to inhibition of signaling pathways to control the function of cellular proteins, thereby interfering with the cellular processes such as apoptosis (Rivas et al., 2001; Nakamura et al., 2001; Lee et al., 2009) proliferation and angiogenesis (Wies et al., 2008; Li et al., 2019; Li et al., 2020). vIRF3 (LANA-2) expression is detected in nearly all virus infected cells in PEL and MCD tumors, and vIRF3 is a bona fide oncogene which can inhibit the function of p53. Moreover, among the KSHV vIRFs, the function of vIRF3 is thought to be B cell-specific. Interestingly, the expression level of vIRF3 does not fluctuate even after lytic reactivation (Rivas et al., 2001), suggesting that there is an alternative level of regulation driving vIRF3 expression in B cells. In latently infected PEL cell lines, vIRF3 is linked to decreased MHC-II expression, and vIRF3 also modulates both type II (Schmidt et al., 2011) and type I interferon responses (Lubyova et al., 2004). vIRF3-mediated inhibition of IFNγ results in inhibition of both PI3 and PI4V promoter of class II transactivator (CIITA) transcription (Schmidt et al., 2011). Importantly, vIRF3 is required for the survival of both EBV co-infected and EBV negative B cell lymphomas in vitro (Wies et al., 2008).

Kaposi’s Sarcoma-Associated Herpesvirus Modulation of B Cell Phenotypes

The Proliferation and Plasmablast Differentiation

PEL is an immunoblastic tumor affecting the pericardial or pleural area of the body cavities. PEL tumor cells are negative for most B cell surface markers except CD138/syndecan, a marker of terminal plasma cell differentiation (Jenner et al., 2003). These terminally differentiated CD138+CD20+ and CD20− plasma cells are highly...
targeted by KSHV infection in primary B cells of tonsillar sample, gaining greater survival rate for CD20− cells over 3 days post infection. This indirect survival effect is as a result of differentiation of other B cell lineages into the CD138+ cells (Aalam et al., 2020). Interestingly, more than 60% of the KSHV infected B cells from PBMCs of KS positive patients are positive for CD138 (Bella et al., 2010). In MCD, the pathological cells are monotypic/polyclonal plasmablasts located in the mantle zone of spleen and lymph nodes (Du et al., 2001). These cells express PRDM1 / BLIMP1 marking them as pre-plasma or terminal plasma stage of B-cell differentiation (Chadburn et al., 2008). Most of KSHV infected B cells in MCD patients express IL-6 (Du et al., 2001), and the importance of IL-6 signaling in MCD is illustrated by the finding that tocilizumab (an IL-6R blocking monoclonal antibody) can ameliorate the symptoms or even lead to prolonged remission in some MCD cases (Song et al., 2010; Galeotti et al., 2012; Ramaswami et al., 2020). In ex vivo infection models, particularly those performed in tonsillar B lymphocytes, the immunophenotype of infected cells closely resembles the pathological cells present in MCD (Du et al., 2001; Chadburn et al., 2008; Totonchy et al., 2018). Latently KSHV infected B cells from the tonsil (characterized by LANA dots), proliferate, and express a high level of IL-6R and CD27 on their surface exhibiting plasma blast morphology at 72 h post-infection (Hassman et al., 2011). Similarly, KSHV infection of naïve B lymphocytes from human tonsil upregulates IL-6 secretion as well as CD27 expression (Totonchy et al., 2018). Ex vivo infection of activated peripheral blood B cells expressing DC-SIGN results in infection of primarily naïve and IgM memory B cells at early times post-infection (Rappocciolo et al., 2008). Remarkably, a similar expansion of MZ-like memory and naïve B cells is seen in PBMC from HIV negative KS patients (Bella et al., 2010). Taken together, the concordance between pre-disease immunophenotypes, ex vivo infection immunophenotypes and the phenotypes seen in KSHV lymphoproliferative diseases suggests that KSHV infection manipulates the B cell compartment toward particular immunophenotypes even in the absence of overt KSHV-associated lymphoproliferative disease and establishing that the Igλ+ phenotype in MCD is driven directly by KSHV infection. Further study is needed to characterize the intervening events that drive KSHV infected B cells from these early manipulations of B cell phenotype and physiology to overt pathological lymphoproliferation.

**DISCUSSION**

KSHV has been co-evolving within the human immune system for thousands of years, and it has developed a plethora of mechanisms for manipulating both B cell physiology and overall immunology which are just beginning to be understood. In recent years, progress on this has been accelerated by new models allowing efficient infection of tonsil-derived primary B cells (Kang and Myoung, 2017). Some of these primary human samples can last up to 10 days, giving ample time to explore the early infection events. One of the hurdles of studying human primary B cell is their limited survival and difficulty of immortalizing them. In the study by Faure et al. (2019), they could achieve up to 20 fold increase in KSHV infection of peripheral B cells co-infected with EBV. These cells were best infected when exposed to KSHV within 24 h of EBV infection and could survive for months under culture conditions. In our recent paper (Aalam et al., 2020), we have generated a library of 40 tonsil specimen and included detailed B cell subtype analysis and de novo infection model. The samples exhibited diverse range of susceptibilities and determined varieties of B cells are susceptible to KSHV infection with CD138+ cells being highly targeted population. However, detailed infection analysis on what drives the susceptibility on these samples are missing as is information about the contributions of cellular and viral genes as well as the immunological milieu to the emergence of pathological lymphoproliferation. While, adherent cells and B cell lines are extensively used for their convenience and ease of manipulation, confirming the same findings within the human primary cells should not be overlooked. Therefore, more systematic and detailed studies are required to evaluate KSHV molecular virology in primary B cells to decode the dynamics of KSHV pathology in the lymphocyte compartment.

**AUTHOR CONTRIBUTIONS**

FA was responsible for content curation and writing of the manuscript. JT was responsible for editing the manuscript, supervision, and obtaining funding. All authors contributed to the article and approved the submitted version.

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Enhancing the Expression of the Master Switch Protein RTA. *PloS Pathog.* 15 (11), e1008160. doi: 10.1371/journal.ppat.1008160

Wies, E., Mori, Y., Hahn, A., Kremmer, E., Stürzl, M., Fleckenstein, B., et al. (2008). The Viral Interferon-Regulatory Factor-3 Is Required for the Survival of KSHV-Infected Primary Effusion Lymphoma Cells. *Blood* 111 (1), 320–327. doi: 10.1182/blood-2007-05-092288

Wu, F. Y., Tang, Q.-Q., Chen, H., ApRhys, C., Farrell, C., Chen, J., et al. (2002). Lytic Replication-Associated Protein (RAP) Encoded by Kaposi Sarcoma-Associated Herpesvirus Causes P21CIP-1-Mediated G1 Cell Cycle Arrest through CCAAT/Enhancer-Binding Protein-Alpha. *Proc. Natl. Acad. Sci. U.S.A.* 99 (16), 10683–10688. doi: 10.1073/pnas.162352299

Zhao, Y., Ye, X., Shehata, M., Dunker, W., Xie, Z., and Karijolich, J. (2020). The RNA Quality Control Pathway Nonsense-Mediated MRNA Decay Targets Cellular and Viral RNAs to Restrict KSHV. *Nat. Commun.* 11 (1), 3345. doi: 10.1038/s41467-020-17151-2

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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