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Animal models of tumorigenic herpesviruses — an update

Dirk P Dittmer, Blossom Damania and Sang-Hoon Sin

Any one model system, be it culture or animal, only recapitulates one aspect of the viral life cycle in the human host. By providing recent examples of animal models for Epstein–Barr virus and Kaposi sarcoma-associated herpesvirus, we would argue that multiple animal models are needed to gain a comprehensive understanding of the pathogenesis associated with human oncogenic herpesviruses. Transgenic mice, homologous animal herpesviruses, and tumorgraft and humanized mouse models all complement each other in the study of viral pathogenesis. The use of animal model systems facilitates the exploration of novel anti-viral and anti-cancer treatment modalities for diseases associated with oncogenic herpesviruses.

Address
Lineberger Comprehensive Cancer Center, Department of Microbiology and Immunology, Center for AIDS Research (CfAR), School of Medicine, University of North Carolina, Chapel Hill, USA

Corresponding author: Dittmer, Dirk P (ddittmer@med.unc.edu)

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Introduction

Herpesviruses are ubiquitous in the human population and establish lifelong persistence in the body. Their evolutionary strategy is to be disseminated through prolonged and intimate contact among their hosts. For this transmission strategy to have evolved, the predominant phenotype of the infected carrier has to subtle — otherwise no other potential host would come close; the most dramatic phenotype must manifest itself only after a long period of asymptomatic shedding, typically after the next generation of hosts has been infected. Indeed, herpesviruses are normally and predominantly 'silently' transmitted from mother to child. Mother-to-child transmission in infancy is the predominant mode for acquiring Epstein–Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV) in endemic healthy populations [1,2].

Cancers associated with these two human viruses manifest themselves only in a minor fraction of infected individuals and only in the context of co-factors that affect the latent reservoirs, for example, malaria-associated B cell activation in the case of EBV. EBV is associated with multiple cancers including Burkitt lymphoma, nasopharyngeal carcinoma (NPC), a subset of gastric cancers, a subset of Hodgkin disease, non-Hodgkin lymphoma localized to the central nervous system, and post-transplant lymphoproliferative disease (PTLD) in the context of iatrogenic immunosuppression. Furthermore, EBV is associated with other non-neoplastic diseases including infectious mononucleosis, oral hairy leukoplakia, lupus [3] and X-linked immunodeficiency [4]. These different EBV disease states represent a variety of infected cell types, gene expression states, and levels of host immune activation. Hence, it would not be possible to recapitulate all these mechanistically different outcomes of infection in just one animal model.

KSHV, also known as human herpesvirus 8, causes Kaposi sarcoma (KS), primary effusion lymphoma, a variant of multicentric Castleman's disease and an acute replication syndrome associated with inflammation [5•]. It is instructive to enumerate the many different scenarios in which KS has been observed, each representing a different host/infection stage.

(a) Classic KS is predominantly a disease of older men and speculatively associated with diminished immune control due to aging. It can also be thought of as the result of an ever-expanding reservoir of latent B cells in response to environmental stimuli. As this latent reservoir increases, so does the likelihood that deleterious mutations occur in the infected B cells.
(b) Iatrogenic KS is the result of chemical immunosuppression. Interestingly, switching from the T cell-selective immunosuppressant cyclosporine/FK506 to the T and B cell immunosuppressant rapamycin/sirolimus is associated with KS regression.
(c) Endemic KS, before the emergence of human immunodeficiency virus (HIV), is a disease of children in a specific geographic locale in Sub-Saharan Africa, which also sees a geographic clustering of malaria and Burkitt lymphoma.
(d) AIDS-KS is associated with diminished immune function due to HIV infection and enhanced KSHV transmission in high-risk populations. Today, almost 20 years after the introduction of anti-retroviral therapy (ART), KS remains the most common cancer

in people living with HIV/AIDS both in Sub-Saharan Africa and in the US/Europe.

(e) KS now also develops in latent HIV-infected patients on long-term ART, that is, in the absence of active HIV replication and despite a reasonable number of CD4 cells (>200 cells/mm^3). These patients tend to be older men and may represent the intersection of incomplete immune repertoire restoration after HIV exposure, long-term immune activation due to microbial translocation, and diminished immune function due to aging.

(f) In Sub-Saharan Africa, KS is also observed in children that acquired both HIV and KSHV at infancy from their mother [1].

(g) Organ and bone marrow transplantation of HIV-positive patients is now routine, and failure rates in ART-adherent recipients are no worse than in HIV-negative patients with comparable co-morbidities. This makes sense intuitively since transplant-associated immunosuppressants suppress replicating CD4 T cells, the preferred vehicle for lytic HIV replication. Some of these transplant recipients or the organ donors also carry KSHV and as a consequence KS may develop.

The above disease types each represent a different genesis from the primary infection event to fulminant disease, each deserving and necessitating a different animal model to capture and recreate the salient features of tumor development.

Kaposi sarcoma-associated herpesvirus (KSHV)

There is no animal model for KSHV. In fact there is no one, perfect model for any human virus. By their nature and design, all models whether a tissue culture model, 3D organ culture, or animal model system, represent one aspect of disease but never the complete human infection cycle. A good animal model is one that faithfully represents a part of the viral life cycle, or a stage of carcinogenesis, for which no other experimental systems exist. A good animal model system is also one that is inexpensive and easy to manipulate. It fills a gap in our understanding of pathogenesis and allows for the testing of anti-viral or anti-cancer agents.

KSHV does not replicate in any species except *Homo sapiens*. Even most primates cannot be productively infected by KSHV. Even in infected humans, KSHV viral loads in plasma are diminutive in comparison to EBV viral loads and those of other herpesviruses. This is perhaps due to the multitude of cell innate restrictions for this virus [6,7]. Models of primary KSHV infection are limited to humanized mouse models [8•]. These serve to reveal cellular tropism (CD19 B cells, macrophages), tissue preference (spleen), viral latency, interactions with other viruses, host immune responses, and sensitivity to replication inhibitors, such as ganciclovir. Viral replication in these models is extremely limited and the input dose is rarely amplified. No serial transmission has been demonstrated among these animals at this time.

KSHV can persist in non-human primates and in rare cases causes KS-like lesions [9]. Again, viral replication is limited and transmission is not observed. For all intents and purposes, non-human primates can be considered dead-end hosts for KSHV.

Non-human rhadinoviruses

In the absence of an infection model for the human virus, homologous viruses and transgenic models have been explored. Each of these mimics different aspects of the disease or the phenotype of a subset of viral gene products. Each of these has been successful and must be considered significant in its own right.

KSHV is part of the rhadinovirus subgroup of gammaherpesviruses. Rhadinoviruses are divided into two lineages (reviewed in [10]). One lineage is represented by KSHV and a primate virus named retroperitoneal fibromatosis herpesvirus (RFHV), and the other lineage is represented by herpesvirus saimiri (HVS) and rhesus monkey rhadinovirus (RRV). RRV has served as a robust animal model system for KSHV. Two independent strains of RRV have been sequenced and their genomes are very similar to each other and to KSHV. RRV replicates to high titers in cell culture and the virus is readily detectable in rhesus macaques. A breakthrough development was the creation of RRV recombination systems, which allowed for viral genetics and the exploration of individual RRV genes in the context of animal infections, as well as for the development of RRV as a vaccine vector [11–13]. In the context of simian immunodeficiency virus (SIV), RRV can induce lymphoma though KS-like skin lesions have not been observed. HVS was the first rhadinovirus isolated from primates and HVS shares molecular mechanisms and host cell targets with KSHV [10,14]; however, this virus infects and transforms T cells in culture. While KSHV has the predilection to establish latency in almost all environments, the primate rhadinoviruses such as RRV and HVS readily enter the lytic replication phase and produce plaques on primary fibroblasts. Unlike KSHV, most primate rhadinoviruses exhibit population seropositivity rates above 80%, i.e. similar to the alpha- and beta herpesviruses.

The mouse homolog of KSHV is murine herpesvirus 68 (MHV68). Before the discovery of KSHV, MHV68 was used as a mouse model for EBV. MHV-68 replicates to high titers in culture and in wild-type mice (lung, spleen), it establishes latency in CD19 B cells and the myeloid compartment, and it can be reactivated from latency. It does not form lymphoma or skin lesions upon natural infection of wild-type mice; however, MHV68 can immortalize and transform fetal liver-derived murine B cells