Commentary

Persistent γ-herpesvirus Infections: What Can We Learn from an Experimental Mouse Model?

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The human γ-herpesviruses, EBV (or HHV-4) and Kaposi's sarcoma-associated herpesvirus (KSHV or HHV-8) are oncogenic viruses that induce a readily controlled lytic infection followed by the establishment of life-long latency. This quiescent state is maintained by the host immune system. In most cases the persistent infection is asymptomatic or accompanied by benign cellular proliferations. However, occasionally persistent γ-herpesvirus infection is associated with the development of malignancies, including Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, Kaposi's sarcoma, and B cell lymphoproliferative syndromes. Most of these malignancies develop after years of viral dormancy, and are associated with viral reactivation. The important role for immune control in preventing the development of malignancies is illustrated by the fact that immunosuppression, as a consequence of disease (AIDS) or posttransplant immunosuppressive therapy, leads to the development of EBV-associated lymphoproliferative syndromes and lymphomas, and KSHV-associated Kaposi's sarcoma (1, 2). Because viral pathology is associated primarily with reactivation of latent virus rather than with the acute infection, it is essential to understand viral mechanisms involved in reactivation from latency, and host mechanisms of immune control.

For the human γ-herpesviruses, most of our knowledge of latent infection has been derived from in vitro studies, usually from cell lines. However, this approach does not allow detection of host/virus interactions in the context of a normal infection in vivo. Because the γ-herpesviruses have coevolved with their host species, they are highly species specific. Whereas some primate species can be infected with EBV, these systems do not mimic natural infections, and are of limited usefulness. SCID mice engrafted with human lymphocytes have been used to study γ-herpesvirus–associated malignancies, but these models have limited utility as models of latent viral infection (3).

An experimental breakthrough came with the isolation of a murine γ-herpesvirus, γHV68 (4). Comparison of the γHV68 genome with other γ-herpesviruses has clearly established γHV68 as a γ-herpesvirus, more closely related to the γ2-herpesviruses such as KSHV than the γ1-herpesviruses, such as EBV (5). Although all the γ-herpesviruses share blocks of conserved genes, there is only limited homology between the genes controlling latency and transformation among the viruses, because each of the viruses is uniquely adapted to its host (5). Despite this, there are striking biological similarities between γHV68 and the human γ-herpesviruses in terms of the establishment and immune control of the acute and latent stages of infection (for reviews, see references 6–9), and γHV68 latency genes have been identified (10–13). γHV68 thus provides a powerful experimental system for studying fundamental aspects of γ-herpesvirus virology, pathology, and immunity in an easily manipulated small animal. Although γHV68 is neither EBV nor KSHV, the information learned with this murine system will undoubtedly be useful, and has already led to a number of new insights into γ-herpesvirus biology and pathogenesis. For example, a novel mechanism of immune evasion, secretion of a broad-spectrum chemokine-binding molecule, has been defined (14, 15). As this protein also binds human CC and CXC chemokines, it may have therapeutic potential (6). In addition, the mouse model has been used to implicate γ-herpesviruses in vascular disease (16–18) and has been used as an experimental model for vaccine development (for a review, see reference 19). Finally, analysis of infection with mutant viruses that lack specific gene function provides a powerful in vivo experimental approach for studying host/virus interactions.

In this issue, Gangappa et al., have taken advantage of the γHV68 model to analyze the in vivo role of two viral gene homologues for cellular genes involved in regulating apoptosis and cell cycle progression (20). Bcl-2 is an anti-apoptotic member of the bcl-2 family and D-cyclin functions in cell cycle progression from G1 to S phase. Due to the lack of appropriate animal models, it had not been possible to determine the function of the v-bcl-2 and v-cyclin genes encoded by the human γ-herpesviruses in vivo. For example, efforts to determine the role of the EBV bcl-2 homologue, BHRF1, in EBV infection by comparing the ability of EBV wild-type and BHRF1-deficient viruses to infect and transform primary lymphocytes, showed no differences (21). Despite this negative result, the finding that BHRF1 was universally present in the EBV genome suggested an essential role. γHV68 was mutated by ho-

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mologous recombination to be deficient in either v-bcl-2 (20) or v-cyclin (22, 23). Analysis of mice infected with the mutant viruses showed that neither v-cyclin nor v-bcl-2 were required for viral replication in vitro or during acute infection in vivo, although the v-cyclin–deficient virus was shown to have a replicative disadvantage following coinfection with wild-type virus (23). Pathological effects of the acute infection, assessed in terms of lethality in immunodeficient mice and lethal meningitis after intracerebral administration to weanling mice, showed no differences between the wild type and mutant viruses (20, 22). In contrast to the lack of an effect in the acute stages of infection, mutation of either v-bcl-2 or v-cyclin prevented reactivation from latency, assessed by ex vivo reactivation assays (20, 22, 23). Viral reactivation is measured by the ability of latently infected cells to spontaneously reactivate and cause cell lysis when plated in vitro on a susceptible cell monolayer. Although it hasn't been directly demonstrated, the assumption is that ex vivo reactivation accurately reflects in vivo viral reactivation. Gangappa et al. took full advantage of the γHV68 mouse model by examining the consequences of chronic infection in immunocompromised mice, so that the pathological effects of viral reactivation in the absence of immune control could be assessed in terms of the development of chronic virus-associated pathology. A major consequence of chronic γ-herpesvirus infection in man is the development of malignancies. Although γHV68 is also associated with increased frequencies of malignancies in mice (24), this isn't an ideal experimental end point, as oncogenesis is an incompletely understood, slowly developing, multi-step process, of which viral infection is only one component. However, persistent γHV68 replication in immunocompromised mice deficient for the IFN-γ receptor (IFNγR-deficient mice) has been shown to result in the development of large vessel arteritis (16, 17). Not only did this and other (18) studies support an etiological role of γ-herpesviruses in the development of human vascular disease, the rapid onset of arteritis provided a predictable and quantitative read-out of chronic γHV68 infection in the mouse. Analysis of immunocompromised mice infected with the v-bcl-2- and v-cyclin–deficient viruses showed markedly reduced pathological consequences of chronic viral replication, assessed by decreased lethality and reduced incidence of arteritic lesions. Infection of immunocompromised IFNγR-deficient mice allowed the consequences of viral reactivation, in this case persistent replication, to be readily observed. In addition, the finding that v-bcl-2 and v-cyclin are not essential for acute viral replication, but are required for reactivation from latency, persistent viral replication, and disease during chronic infection, allowed the authors to conclude that persistent viral replication was a consequence of reactivation from latency, rather than cellular transmission of lytic virus, and that different viral genes are required for acute and persistent replication.

γHV68, EBV, and KSHV all coopt the function of cellular bcl-2 and D-cyclin, but each virus has adapted the cellular function uniquely. Members of the bcl-2 family contain up to four bcl-2 homology domains, which are required for heterodimer formation and interaction with other cellular proteins to generate pro- and antiapoptotic molecules. γ-herpesvirus bcl-2 homologues mimic the antiapoptotic functions, but not the pro-apoptotic functions of the cellular bcl-2 family by conserving only the BH1 and BH2 domains (EBV and KSHV) or just the BH1 domain (γHV68) (25, 26). There are also important differences among the γ-herpesviruses regarding the mechanisms by which they coopt the function of the mammalian D-type cyclins (D1, D2, and D3). EBV does not encode a cyclin homologue. Rather, EBV infection, through expression of LMP-1 up-regulates expression of the host cyclin D2. KSHV and γHV68 both contain open reading frames predicted to encode proteins homologous to the mammalian D-type cyclins. The highest level of sequence conservation is within the cyclin box, a domain essential for cyclin-depen-

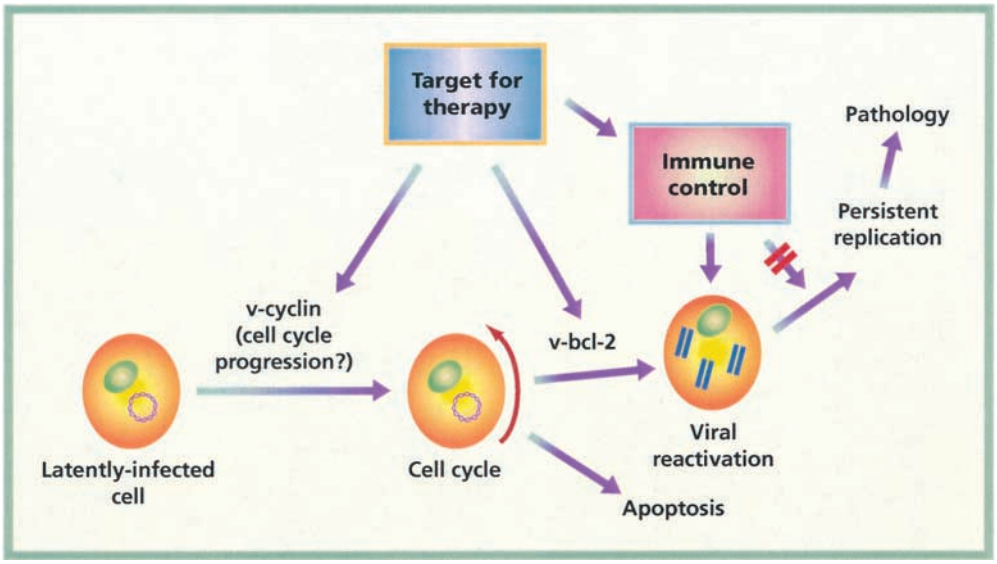

Figure 1. MHV-68 reactivation from latency is dependent upon both v-cyclin and v-bcl-2. A likely scenario is that v-cyclin allows cell cycle progression that is essential for the initiation of viral replication, and that v-bcl-2 prevents the subsequent apoptosis of the cycling cell. In an immunocompetent mouse, viral recrudescence and spread is controlled by the host immune system. However, in the absence of effective immune control, persistent replication leads to pathology. Important targets for immunotherapy include the viral bcl-2 and cyclin homologues and antiviral immune function.

dent kinase binding. The viral proteins do not, however, exactly mimic the function of their host counterparts. For example, the specificity of binding of v-cyclin to cyclin-dependent kinases is relaxed, and normal regulation is disrupted, perhaps due to structural differences between the viral and cellular cyclins.

The similar phenotype of the γHV68 mutant viruses deficient in v-bcl-2 and v-cyclin suggests that regulation of apoptosis and cell cycle are both important for reactivation from latency. Latently infected cells appear to be arrested at a specific point in their cell cycle. Therefore, the requirement for v-cyclin for reactivation from latency is consistent with the need for cell cycle progression to allow efficient DNA replication during virus reactivation. However, viral reactivation and/or cell cycle progression trigger viral or host apoptotic pathways, so there is an additional requirement for antiapoptotic mechanisms, provided by v-bcl-2. A delicate balance between cell cycle progression and prevention of apoptosis must be maintained, and disruption of this balance may contribute to the development of malignancies. Virus reactivation is presumably controlled by the host immune system in immunocompetent individuals. Immune control of the acute lytic infection has been well characterized (6, 7), but less is known about immune control of reactivation from latency. Control of virus reactivation is likely to be mediated by distinct mechanisms, including not only $CD8^+$ T cells, but also IFN-γ (27) and antibody (28).

The link between v-cyclin and v-bcl-2 expression and viral reactivation from latency and pathology established by Gangappa et al., suggest that the γ-herpesvirus–encoded v-cyclin and v-bcl-2 are important targets for therapeutic intervention (Fig. 1). One straightforward approach might be antisense therapy. In this regard, antisense therapy in oncology is undergoing a renaissance, and encouraging results are being obtained with "second-generation" antisense oligonucleotides, including bcl-2, in conjunction with chemotherapy in control of a variety of malignancies (29). Feasibility is further established by the observation that antisense oligonucleotides specific for v-bcl-2 encoded by EBV (BHRF1) effectively inhibit antiapoptotic function (30). Targeting the viral genes would allow early intervention, to prevent viral reactivation and the onset of malignancies in immunosuppressed patients. Preventative intervention is particularly indicated for controlling the development of malignancies in high risk scenarios specifically associated with viral reactivation, such as AIDS patients or after immunosuppressive transplant therapy. In addition, if the association between viral reactivation and vasculitis and atherosclerosis in the mouse is borne out in the human, ultimately such approaches might be useful in the prevention and/or control of human vascular disease. However, it is clear that usurping cellular control of apoptosis by the γ-herpesviruses is more complex than the expression of v-bcl-2. In addition to encoding a v-bcl-2, EBV proteins EBNA-4 and LMP-1 up-regulate transcription of cellular bcl-2 and family members (31–33), LMP-1 interacts with TRAFs and TRADD (34), and E1B19K interacts with the proapoptotic protein, Bax (35). Recently, expression of an EBV-bcl-2 antagonist, BALF1, has been reported (36). Likewise, KSHV encodes other inhibitors of apoptosis, including v-FLIP, which is an inhibitor of Flice (37). Thus, therapeutic targeting of v-bcl-2 might be only partially effective, or may require combination treatment with several oligonucleotides.

A second target for therapeutic intervention is enhancement of immune function. The effectiveness of this strategy is illustrated by the success with adoptive transfer of EBV-specific cytolytic T cells as therapy for EBV-associated lymphoproliferative disorders, which are complications of bone marrow transplantation (38). In addition, we have recently demonstrated an important role for humoral immunity in control of γ-herpesvirus latency (28), supporting the rationale for therapeutic passive transfer of immune serum, which is being addressed systematically in clinical trials (39, 40). Additional therapeutic targets might include enhancement of the antiviral IFN-γ response (20).

In summary, although there are important differences in the murine and human γ-herpesviruses, the availability of the γHV68 mouse model is a major break-through in the field, and it's clear that much can be learned from appropriate interpretation of in vivo studies with this model. The current studies make important progress in understanding mechanisms involved in reactivation from latency, a key step in chronic viral pathogenesis. In addition, γHV68 infection of immunodeficient mice provides an important experimental system for testing new therapeutic approaches for controlling persistent viral replication and pathology (Fig. 1).

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