

## Gamma-Herpesvirus Latency

with

Marcia A. Blackman, Ph.D., Trudeau Institute,  
154 Algonquin Avenue, Saranac Lake,  
NY 12983, USA

MBlackman@trudeauinstitute.org



### Is the mouse $\gamma$ -herpesvirus a good model for the human $\gamma$ -herpesviruses?

**Marcia A. Blackman:** The human  $\gamma$ -herpesviruses, including Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), are large oncogenic viruses. They establish both a lytic infection, which is readily eliminated by the host immune system, and a latent infection, which persists for the life of the host. The virus is normally maintained in a quiescent state by the immune system but, in some cases, is associated with a variety of malignancies, including Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma, B cell lymphoproliferative syndromes, and Kaposi's sarcoma. The  $\gamma$ -herpesviruses have co-evolved intimately

with their host, and thus are highly species-specific. Even the primate species that can be infected with EBV are not faithful models of human infection. Thus, much of what we know about the human viruses has been from *in vitro* studies. In order to dissect host/virus interactions, a model of natural infection is essential. The murine  $\gamma$ -herpesvirus, MHV-68 or  $\gamma$ HV68, therefore provides an important *in vivo* model, particularly for studying the initial stages of infection, including events involved in the establishment of latency and initiation of the host immune response, as well as viral immune evasion mechanisms and vaccination strategies (1).

### What cell types does the virus infect?

**Marcia A. Blackman:** The  $\gamma$ -herpesviruses establish a lytic infection in epithelial cells and a latent infection in a variety of cell types. The major reservoir for EBV latency is the memory B cell, whereas  $\gamma$ HV68 is more promiscuous and establishes latent reservoirs not only in memory B cells, but also in macrophages and dendritic cells (2,3). We have recently shown that B cells and dendritic cells are early targets of  $\gamma$ HV68 infection, with latency being established concurrently with the lytic infection in the lung (4,5). The finding that dendritic cells are an early target of viral

infection is of particular interest since these cells are the primary antigen presenting cell for initiating an immune response. Thus, it is possible that infection of dendritic cells is an immune evasion strategy focused on usurping dendritic cell function. Importantly, we recently showed that whereas *in vitro*  $\gamma$ HV68 infection of dendritic cells had no direct effect on maturation and did not induce cytokine production, infected dendritic cells had altered patterns of cytokine expression upon exogenous activation with LPS, the most striking effect being enhanced IL-10 production (5).

### How important is IL-10 as an immune evasion strategy?

**Marcia A. Blackman:** EBV encodes a viral homologue of IL-10, vIL-10, which has been shown to retain some, but not all, functions of cellular IL-10. Based on *in vitro* studies, the function of vIL-10 *in vivo* is thought to be to suppress antiviral immune responses and promote B cell survival and the establishment of latency (6). This homologue is missing in KSHV and  $\gamma$ HV68, but, as mentioned above, we have recently shown that  $\gamma$ HV68-infected dendritic cells produce elevated levels of (cellular) IL-10 upon exogenous stimulation (5). Consistent with a role of virally-induced IL-10 in immune evasion, it had been reported that latency was reduced in IL-10-deficient mice (7). We confirmed and extended these studies and showed that indeed, despite no effect on the lytic phase of the infection,

both the peak levels of latency and the long-term latent load was reduced in IL-10-deficient mice. In addition, dendritic cells isolated *ex vivo* from infected wild-type mice express elevated levels of IL-10 message. Additional *in vitro* studies showed that viral infection of dendritic cells didn't interfere with antigen processing and presentation, but did interfere with the ability to stimulate normal T cells in a mixed lymphocyte reaction. The defective *in vitro* T cell stimulation was partly ameliorated by neutralizing antibodies to IL-10. Taken together, the data suggest that  $\gamma$ HV68 infection of dendritic cells is a mechanism of viral immune evasion, mediated in part by IL-10 (5). The value of the  $\gamma$ HV68 model in studying dendritic cell infection as an immune evasion mechanism has only begun to be exploited.

### Can vaccination prevent or control latency?

**Marcia A. Blackman:** Despite a vigorous immune response to the initial  $\gamma$ -herpesvirus infection and clearance of lytic virus, latency is established. The latent phase of infection normally remains quiescent in immunocompetent individuals. However, the virus is associated with the development of a variety of malignancies, and reactivation of latent virus during therapeutic or disease-associated immunosuppression often results in lymphoproliferative disease. It is clear that the presence of latent virus poses a significant clinical threat, which could be prevented by sterilizing immunity. What is not clear however is whether effective prophylactic vaccination strategies against the  $\gamma$ -herpesviruses can be achieved, or whether the multiple viral immune evasion mechanisms will always allow latent virus to sneak through and establish a foothold in the host. Early attempts at epitope-based strategies showed that the vacci-

nation effectively reduced the lytic viral load, but had little impact on long-term viral latency (8,9). More recently, it has been shown that vaccination with reactivation-deficient mutant viruses significantly lowers long-term latency following challenge with wild-type viruses (9,10). However, to be effective, the immunity must be sterilizing, as the presence of even dramatically-reduced levels of latent virus poses a potential oncogenic threat. Finally, post-exposure vaccination strategies to boost immunosurveillance by T cells have been tested in the mouse model, but have not yet achieved long-term control of reactivating virus (11). The question of whether vaccination can prevent the establishment of latency or boost immune control of the latent phase of infection remains unanswered. Studies with  $\gamma$ HV68 provide an important experimental model for "proof of principal" testing of vaccination strategies.

### REFERENCES

1. Blackman MA et al. J Exp Med 195, 29, 2002
2. Flaño E et al. J Immunol 165, 1074, 2000
3. Flaño E et al. J Exp Med 196, 1363, 2002
4. Flaño E et al. J Immunol 174, 4972, 2005
5. Flaño E et al. J Immunol 175, 3225-34

6. Nicholas JJ Interferon Cytokine Res 25, 373, 2005
7. Peacock JW et al. Immunology 104, 109, 2001
8. Woodland DL et al. Viral Immunology 14, 217, 2001
9. Stevenson PG et al. J Virol 77, 2522, 2003
10. Tibbetts SA et al. J Virol 77, 2522, 2003
11. Belz GT et al. PNAS 97, 2725, 2000