

Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes**Project: Murine Gammaherpesvirus 68 (MHV-68): a Mouse Model for Studying Gammaherpesvirus Pathogenesis**

Heiko Adler - GSF-National Research Center for Environment and Health, Neuherberg - h.adler@gsf.de

Introduction

Gammaherpesviruses are lymphotropic viruses which establish lifelong infections in their hosts and are associated with cellular transformation and the development of malignancies, particularly in immunosuppressed individuals, for example AIDS patients (Rickinson and Kieff, 2001; Chang et al., 1994; Cesarman and Knowles, 1999). Until recently, mechanistic studies of the role of immunity in gammaherpesvirus infection have been hampered by the species specificity of the human herpesviruses, Epstein-Barr virus (EBV) and human herpesvirus-8 (HHV-8), and the difficulties and costs inherent in analyzing pathogenesis, tumor induction, and mechanisms of immunity in primate models. Infection of laboratory mice with murine gammaherpesvirus-68 (MHV-68) provides a small animal model for addressing basic issues in gammaherpesvirus pathogenesis and immunity (Virgin and Speck, 1999; Speck and Virgin, 1999; Simas and Efsthathiou, 1998). MHV-68 was originally isolated from the bank vole (*Clethrionomys glareolus*) in Slovakia and is a natural pathogen for mice (Blaskovic et al., 1980) (Fig. 1). The overall similarities of MHV-68 to other gammaherpesviruses in terms of genomic organization, pathological symptoms and the establishment of both lytic and latent infection make it a valuable model.



Fig 1: The bank vole (*Clethrionomys glareolus*) is a natural host of MHV-68.

Herpesviruses have double-stranded DNA genomes of a size of 100 to 250 kbp, consisting of up to 225 open reading frames (ORFs) (Roizman and Pellett, 2001). These large and complex genomes can code for an extensive repertoire of gene functions required to efficiently interfere with the host immune response. Although the complete sequence of the genome of many herpesviruses has been determined, the precise function for many of the viral genes, both in vitro, and in particular in vivo, has not been elucidated. Knowing the function of individual viral genes or families of viral genes is important: I) to understand their role in pathogenesis; II) to examine their potential as a therapeutic target; III) for the

rational design of vaccines; and IV) for utilization as a tool in gene therapy (Adler et al., 2003).

In principle, the strategy to assign a function to a viral gene in the context of the whole viral genome is mutagenesis of the gene of interest, followed by the analysis of the phenotype of the mutant virus both in vitro and in vivo. Conventional methods for mutagenesis of herpesviruses included chemical mutagenesis, site-directed mutagenesis by homologous recombination in eukaryotic cells, and manipulating virus genomes using overlapping cosmid clones [reviewed, for example, in (Brune et al., 2000; Wagner et al., 2002)]. These methods proved very useful for the generation of a variety of mutants but their construction is often inefficient, laborious and time-consuming, mainly due to the large genome size and, in case of the beta- and gammaherpesviruses, the slow replication kinetics or the lack of replication in vitro. Conventional methods are performed in eukaryotic cells and therefore difficult to control. One problem is that the analysis of the mutant viral genomes is only possible at the very end of the lengthy experimental procedure and thus additional changes in the viral genome like deletions, rearrangements or illegitimate recombinations are frequently only observed then. Furthermore, the generation of a viral mutant requires selection against nonrecombinant wildtype virus and finally separation of the mutant virus from the wildtype virus, for example by plaque-purification. Additionally, complementing cell lines are required when mutations are introduced into essential viral genes. The construction of a mutant is therefore often a limiting step for the analysis of a viral gene function in the genomic context (Adler et al., 2003).

A completely new approach for the construction of herpesvirus mutants is based on cloning of the virus genome as a bacterial artificial chromosome (BAC) in *E. coli* (Messerle et al., 1997). This technique allows the maintenance of viral genomes as a BAC in *E. coli* and the reconstitution of viral progeny by transfection of the BAC plasmid into eukaryotic cells. Mutagenesis of the virus genome in *E. coli* using prokaryotic recombination functions is possible, thereby allowing the generation of mutant viruses. Using this method, any genetic modification should be possible, thereby facilitating the analysis of herpesvirus genomes cloned as infectious BACs.

Project Status

In our previous research, we have cloned the MHV-68 genome as an infectious bacterial artificial chromosome (BAC) (Adler et al., 2000; Adler et al., 2001). Using this technique, we want to elucidate the role of host and viral genes in the virus-host interaction. So far, using targeted mutagenesis of the MHV-68 BAC, we have constructed and analyzed several MHV-68 mutants (Lee et al., 2003; Simas et al., 2004; Stewart et al., 2004). In the following chapter, one example of our current work, namely investigations on the role of a gammaherpesviral internal repeat for the pathogenesis, is described.

The genomes of gammaherpesviruses contain variable numbers of internal repeats whose precise role for in vivo pathogenesis is not well understood. We used infection of laboratory mice with murine gammaherpesvirus 68 (MHV-68) to explore the biological role of the 40 bp internal repeat of

MHV-68. Using BAC-technology, we constructed several mutant viruses partially or completely lacking this repeat. Both in vitro and in vivo, the loss of the repeat did not affect lytic replication of the mutant viruses when compared to wildtype virus. However, the extent of splenomegaly (splenic mononucleosis), which is associated with the establishment of latency, was reduced. Interestingly, despite the observed reduction of splenomegaly, the frequency of latently infected splenocytes, as determined by ex vivo reactivation assays, was only slightly (3-fold) reduced, and there was no significant difference in the genomic load. Since the 40 bp repeat is a part of the potential open reading frame (ORF) M6, it might function as part of the ORF or as an independent structure. To differentiate between these two possibilities, we constructed several N-terminal mutants of ORF M6, leaving the repeat structure intact but rendering ORF M6 unfunctional. Disruption of ORF M6 did neither affect the lytic nor the latent infection. In ongoing experiments, we try to elucidate the biological mechanisms underlying the observed phenotypes. Our findings suggest that the 40 bp repeat is dispensable for lytic replication but has a role in virus-induced mononucleosis (This work has been recently presented at the 30th International Herpesvirus Workshop, July 30 - August 5, 2005, Turku, Finland).

Outlook

Using targeted mutagenesis of the MHV-68 BAC, we continue to construct MHV-68 mutants. Using these mutants, we will study the role of the targeted genes in the virus-host interaction both in vitro and in vivo. The analysis of MHV-68 mutants will significantly contribute to the understanding of viral gene functions and to the evaluation of their role in the pathogenesis. By using various combinations of virus mutants and mouse mutants, we will attempt to identify both viral and host factors involved in the pathogenesis of gammaherpesvirus infections (Fig.2).

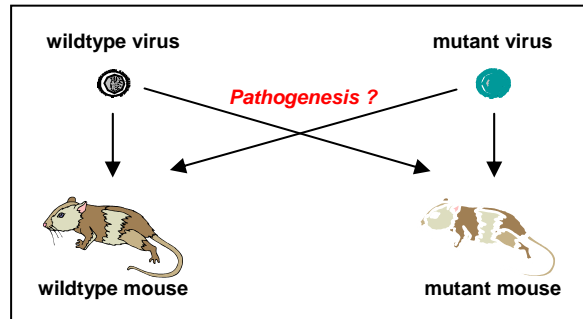


Fig 2: By using combinations of virus mutants and mouse mutants, both viral and host factors involved in the pathogenesis of gammaherpesvirus infections may be identified.

Lit.: 1. Adler,H., Messerle,M., and Koszinowski,U.H. (2001). Virus reconstituted from infectious bacterial artificial chromosome (BAC)-cloned murine gammaherpesvirus 68 acquires wild-type properties in vivo only after excision of BAC vector sequences. *J. Virol.* 75, 5692-5696. 2. Adler,H., Messerle,M., and Koszinowski,U.H. (2003). Cloning of herpesviral genomes as bacterial artificial chromosomes. *Rev. Med. Virol.* 13, 111-121. 3. Adler,H., Wagner,M., and Koszinowski,U.H. (2000). Cloning and mutagenesis of the murine gammaherpesvirus 68 genome as an infectious bacterial artificial chromosome. *J. Virol.* 74, 6964-6974. 4. Blaskovic,D., Stancekova,M., Svobodova,J., and Mistrikova,J. (1980). Isolation of five strains of herpesviruses from two species of free living small rodents. *Acta Virol.* 24, 468. 5. Brune,W., Messerle,M., and Koszinowski,U.H. (2000). Forward with BACs - new tools for herpesvirus genomics. *TIG* 16, 254-259. 6. Cesarman,E. and Knowles,D.M. (1999). The role of Kaposi's sarcoma-

associated herpesvirus (KSHV/HHV-8) in lymphoproliferative diseases. *Semin. Cancer Biol.* 9, 165-174. 7. Chang,Y., Cesarman,E., Pessin,M.S., Lee,F., Culpepper,J., Knowles,D.M., and Moore,P.S. (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's Sarcoma. *Science* 266, 1865-1869. 8. Lee,B.J., Koszinowski,U.H., Sarawar,S.R., and Adler,H. (2003). A gammaherpesvirus G protein-coupled receptor homologue is required for increased viral replication in response to chemokines and efficient reactivation from latency. *J. Immunol.* 170, 243-251. 9. Messerle,M., Crnkovic,I., Hammerschmidt,W., Ziegler,H., and Koszinowski,U.H. (1997). Cloning and mutagenesis of a herpesvirus genome as an infectious bacterial artificial chromosome. *Proc. Natl. Acad. Sci. USA* 94, 14759-14763. 10. Rickinson,A.B. and Kieff,E. (2001). Epstein-Barr Virus. In *Fields - Virology*, D.M.Knipe, P.M.Howley, D.E.Griffin, M.A.Martin, R.A.Lamb, B.Roizman, and S.E.Straus, eds. (Philadelphia: Lippincott Williams & Wilkins), pp. 2575-2627. 11. Roizman,B. and Pellett,P.E. (2001). The Family Herpesviridae: A brief introduction. In *Fields - Virology*, D.M.Knipe, P.M.Howley, D.E.Griffin, M.A.Martin, R.A.Lamb, B.Roizman, and S.E.Straus, eds. (Philadelphia: Lippincott Williams & Wilkins), pp. 2381-2397. 12. Simas,J.P. and Efsthathiou,S. (1998). Murine gammaherpesvirus 68: a model for the study of gammaherpesvirus pathogenesis. *Trends in Microbiology* 6, 276-282. 13. Simas,J.P., Marques,S., Bridgeman,A., Efsthathiou,S., and Adler,H. (2004). The M2 gene product of murine gammaherpesvirus 68 is required for efficient colonization of splenic follicles but is not necessary for expansion of latently infected germinal centre B cells. *J. Gen. Virol.* 85, 2789-2797. 14. Speck,S.H. and Virgin,H.W.IV. (1999). Host and viral genetics of chronic infection: a mouse model of gamma-herpesvirus pathogenesis. *Current Opinion in Microbiology* 2, 403-409. 15. Stewart,J.P., Silvia,O.J., Atkin,I.M., Hughes,D.J., Ebrahimi,B., and Adler,H. (2004). In vivo function of a gammaherpesvirus virion glycoprotein: influence on B-cell infection and mononucleosis. *J. Virol.* 78, 10449-10459. 16. Virgin,H.W.IV. and Speck,S.H. (1999). Unraveling immunity to gammaherpesviruses: a new model for understanding the role of immunity in chronic virus infection. *Curr. Opin. Immunol.* 11, 371-379. 17. Wagner,M., Ruzsics,Z., and Koszinowski,U.H. (2002). Herpesvirus genetics has come of age. *Trends in Microbiology* 10, 318-324.