REVIEW

Of Mice and Men: Murine Gammaherpesvirus 68 as a Model

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Introduction

Much is known about the infection of cells *in vitro* by EBV and KSHV/HHV 8 (1,2). However, the study of their biology and pathogenesis in the host has been restricted. Direct studies in human subjects are complicated. Also, both viruses have extremely restricted host ranges and even within susceptible non-human primates, infection does not recapitulate key aspects of natural infection (3). The comparative analysis of other gammaherpesvirus infections may therefore give valuable insight into mechanisms operating during EBV and KSHV/HHV 8 infection and a means of testing therapeutic strategies. A potentially useful model system, especially for the study of fundamental aspects of host control, is the experimental infection of laboratory mice with the murine gammaherpesvirus, MHV-68.

The virus

MHV-68 is a natural pathogen of free-living murid rodents (4). A great advantage of MHV-68 is that it will undergo productive replication, form plaques and grow to high titres in conventional cell monolayer cultures such as BHK or 3T3 (5-7). MHV-68 will not transform primary cells (BM Dutia, unpublished observations) but will infect and persist in B lymphocyte cell lines (8). The prime example is S11 that outgrew from an MHV-68-positive B lymphoma (9). This line is analogous to EBV-positive Burkitt's lymphoma lines (10) and KSHV/HHV 8-positive body cavity B lymphoma lines (2). Thus, S11 cells carry MHV-68 predominantly in a latent form with 1-5% of cells sustaining productive replication.

The MHV-68 genome has 118 Kb of largely unique DNA flanked by terminal repeats (11). Of the 80 or so open-reading frames (ORFs), most are homologous to other gammaherpesvirus genes (12-14). However, there are a number of ORFs, termed M1-14 that have no obvious relationship to known virus or cellular genes (14). Of note is the fact that there are no homologues of the EBV latency-associated genes (EBERs, EBNAs, BARF0 and LMPs) or the unique KSHV/HHV 8 genes (K1-15). The exact mechanisms involved in the establishment and maintenance of latency are therefore clearly different for MHV-68. There is, however, a block of four ORFs (cyclin D, bcl-2, ORF 73 [LANA], IL-8 receptor) having similarity to EBV and KSHV/HHV 8 genes that may be important in pathogenesis. These MHV-68 genes will be of obvious interest and importance and at least one (bcl-2) has already been shown to be functional (DJ Roy and JP Stewart, unpublished observations). At the left-hand end of the genome are eight transfer RNA (tRNA)-like genes (15). The biological significance of these tRNAs is unclear as they do not function as tRNAs and at least four can be deleted without discernible effect (16). However, the tRNAs have a similar pattern of expression to the EBER genes and are therefore important tools in identifying latently infected cells (15,17,18).

Infection and pathogenesis

Transmission of gammaherpesvirus infections involves close contact, probably occurring via either oral or respiratory routes (1). We therefore feel that in an experimental context, intranasal infection of mice by MHV-68 represents the most authentic route. Mice can be inoculated via the intravenous or intraperitoneal routes; however, the course of infection in these cases is very different and it is therefore hard to see how these methods may relate to a natural challenge (19). Mice inoculated intranasally with MHV-68 establish productive infection in the lung in alveolar epithelial cells causing an interstitial and peri-bronchiolar pneumonia (7,20,21). This productive

phase of infection lasts for around 10 days, during which period the virus spreads via lymph nodes to the spleen (7). Here it causes an acute, transient splenomegaly and establishes a readily reactivatable latency in B cells in germinal centres (18,22-24), although recent work has shown latent infection of other cell types such as macrophages (25). Latently infected cells appear in the first week post-infection, reach peak levels during the second to third week before declining to a basal level (1 in 10^6 leukocytes) that is maintained indefinitely in the host (7,22). In C57BL/6 mice, mononucleosis occurs during the third week of infection and is characterised by an expansion in $V\beta4^+$ $CD8^+$ T cells raising the possibility of the presence of a virus super-antigen (26).

An important recent finding is that the spleen is not the only site of latency and that MHV-68 also persists indefinitely in lungs (23). Here, alveolar epithelial cells carry the virus in a latent form (17). This raises the possibility that lungs may be a reservoir for EBV and KSHV/HHV 8, as has been previously suggested (27). Also, since both EBV and KSHV/HHV 8 have been associated with chronic lung disease (28-30), chronic MHV-68 infection may prove useful in modelling these diseases.

A common feature of gammaherpesvirus infection is the genesis of lymphoproliferative disorders. MHV-68 infection leads to lymphoma induction in mice (31). While virus is associated with the tumours and one virus-positive B lymphoma line has been grown out (9), the exact relationship between the virus and tumour induction is still under investigation.

Gene expression during acute infection and persistence

The study of MHV-68 lytic gene expression is relatively easy due to its ability to productively infect cell culture cells. There is a conventional cascade of gene expression and immediate-early (e.g. ORF 50 [BRLF1 homologue], ORF 73 [LANA]), early (e.g. thymidine kinase) and late (e.g. glycoproteins B, H and 150) genes have been identified (S Selvarajah, BM Dutia, DJ Roy and JP Stewart, unpublished observations) (32-34). Progression is rapid, late gene expression can be seen at 8 hours post-infection and the infectious cycle is completed within 24 hours (33,34).

Determination of latency-associated genes has been greatly aided by the development of the S11 lymphoma line (9). All proven latency-associated transcription yet found, centres around the most leftward 8 Kb of the genome. The tRNAs and the M2 gene are expressed in latently infected S11 cells and their expression persists *in vivo* (18,35,36). In contrast, the M3 gene is not expressed in latently infected S11 cells but is expressed in the spleen (18,36). Thus, like EBV, there may well be multiple forms of MHV-68 latency. In addition, there are also multiple spliced transcripts expressed in S11 cells crossing the terminal repeats (36). However, the complete cDNA and ORF associated with these has yet to be determined. Recent RT-PCR data has suggested that other genes (bcl-2, ORF73 and IL-8 receptor) were transcribed during latency (35). However, these are all known lytic cycle genes and thus the association of these genes with latent infection has yet to be conclusively proven (DJ Roy, M Wakeling, JP Stewart, unpublished observations).

Immunological events during infection

Acute MHV-68 infection in the lung induces a typical inflammatory response consisting mainly of mononuclear phagocytes and lymphocytes (7). Both directly cytolytic and interferon-γ (IFN-γ) -secreting, virus-specific $CD8^+$ T cells are found and depletion experiments have shown that these cells are the critical element in controlling acute infection (37,38). Thus, while antivirus $CD4^+$ T cells and antiviral antibody are found, a lack of either or both of these only causes a minor delay in virus clearance (23,24,37-40).

Latently infected B cells in the spleen initiate splenomegaly since it does not occur in transgenic B cell-deficient mice (23,24). B cells have also been shown to be important in trafficking the virus to the spleen (17). Spleen cell numbers double during splenomegaly, with an increase in both B cells and T cells (41). Unlike EBV, there is no evidence to suggest that MHV-68 directly drives B cell proliferation by means of 'transformation' (BM Dutia, unpublished observations). We do know, however, that $CD4^+$ T cells are critical for the rapid expansion of infected B cells and that elevated levels of IL-6, IL-10 and IFN-γ are detected during splenomegaly (41-44). It seems likely, therefore, that infected B cells cause activation of $CD4^+$ T cells either by cognate interactions or by expression of a super-antigen (26,41,45). T cell 'help' in turn leads to B cell expansion and splenomegaly ensues via this positive feedback loop. As in the lung, either depletion of $CD8^+$ T cells or the infection of mice deficient in $CD8^+$ T cells has shown that these cells clearly influence the control of acute infection in the spleen (24,40,46). In addition, transgenic mice deficient in the IFN-γ receptor have a peak level of latently infected B cells that is 10 times higher. Destruction of spleen architecture and fibrosis ensue indicating a role for IFN-γ in effector mechanisms involved in the initial control of latently infected B cells (47).

Immunity to MHV-68 persists indefinitely at a background level and is important in controlling reactivating virus (37,39,40). Interestingly, evidence suggests that $CD8^+$ T cells, $CD4^+$ T cells and antibody are all individually capable of controlling virus reactivation (17,40). The persistence of latently infected lung and spleen cells in the face of the continued presence of virus-specific $CD8^+$

cytotoxic T cells implies that while some infected cells are sensitive to T cell recognition, others evade this effector response. There may clearly be parallels with EBV where type III latency is targeted by T cells, but type I latency evades recognition (10,48). However, the mechanisms involved here are clearly of seminal importance to our understanding gammaherpesvirus persistence, and the MHV-68 model provides the ideal means with which to investigate this question further.

While the adaptive immune response and in particular $CD8^+$ T cells are clearly important for the control of gammaherpesvirus infections, work with MHV-68 has shown that innate defences, in particular type I (α/β) interferons, also play a critical role. Infection of transgenic mice with a lesion in the type I interferon receptor gene resulted in 90% mortality, whereas wild-type mice showed a 100% recovery from the same dose of virus (BM Dutia, unpublished observations) (46,49). Death occurred in the type I receptor-deficient mice after 6-8 days as a result of overwhelming virus replication in the lung. It therefore appears that type I interferons play a crucial role in controlling the initial virus replication and their role in gammaherpesvirus-associated disease warrants further investigation.

Development of therapeutic strategies

An important aspect of the development of MHV-68 as a model is the ability to test strategies for prophylactic and therapeutic intervention against EBV and KSHV/HHV 8 infection and disease. These include investigating the effects of established and novel antiviral drugs, vaccination and other immunotherapies such as administration of antivirus antibody or cytotoxic T cells.

Like EBV, MHV-68 expresses a thymidine kinase that will activate antiviral drugs such as acyclovir (32,50). Several groups have studied the effects of this and other drugs on the course of infection of mice by MHV-68 (50-52). While these compounds were generally effective at inhibiting virus replication in the lungs, prophylactic treatment was not effective at arresting the establishment of a latent infection. Future studies, particularly in immunocompromised animals, will provide insight into the role that these and other novel drugs could play in the therapy of EBV and KSHV/HHV 8 infections of man.

Much emphasis has been placed on the potential of vaccinating against EBV to protect not only against infectious mononucleosis but also against virus-associated tumours (3). The major candidate for an EBV vaccine antigen is gp350/220 since this protein mediates binding of EBV to B cells, and antibodies to this protein neutralise virus infectivity. Similarly, MHV-68 particles contain an equivalent glycoprotein termed gp150 and antibodies to gp150 also neutralise virus infectivity in the absence of complement (34). In preliminary studies to model the efficacy of vaccinating against EBV with gp340/220, a recombinant vaccinia virus expressing MHV-68 gp150 was produced (53). Immunisation with this recombinant induced a virus-neutralising antibody response and after challenge with MHV-68, mice were protected from the rapid rise in latently infected B cells and splenomegaly (equivalent to mononucleosis). However, MHV-68 latency in B cells was ultimately established. This implies that while a major pathology associated with gammaherpesvirus infection can be regulated by vaccination, other strategies such as inclusion of latency-associated antigens may be required to protect against the establishment of latency. The recent discovery of MHV-68 latency-associated genes such as M2 and the identification of this as a target for $CD8^+$ cytotoxic T cells (36) means that such general strategies for vaccination as well as T cell immunotherapy can now be tested in this model system.

Summary

Development of MHV-68 in the mouse as an amenable small animal model system has clearly enabled the study of key aspects of gammaherpesvirus biology in the context of a natural host. One considerable advantage of the system is the capacity to harness the power of transgenic 'knockout' technology to study the relative contributions of host determinants in pathogenesis. The impact of this on our understanding of gammaherpesvirus biology is already considerable, particularly in relation to components of the immune system involved in antiviral defence. An obvious and significant use of this model will be in the exploration of therapeutic strategies to target latent infections and their associated diseases.

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References

1. Rickinson AB, Kieff E. Epstein-Barr virus. In: Fields BN, Knipe DM, Howley PM, editors. Field's Virology. New York: Lippincott-Raven, 1996:2397-446.
2. Schulz TF. Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8). *J Gen Virol* 1998;79:1573-91.
3. Wolf HJ, Morgan AJ. Epstein-Barr virus vaccines. In: Medveczky PG, Friedman H, Bendinelli M, editors. Herpesviruses and Immunity. New York: Plenum Press, 1998:231-41.
4. Blaskovic D, Stancekova M, Svobodova J, Mistrikova J. Isolation of five strains of herpesviruses from two species of free living small rodents. *Acta Virol* 1980;24:468.

5. Svobodova J, Blaskovic D, Mistrikova J. Growth characteristics of herpesviruses isolated from free living small rodents. *Acta Virol* 1982;26:256-63.
6. Ciampor F, Stancekova M, Svobodova J, Mistrikova J. Electron microscopy of rabbit embryo fibroblasts infected with herpesvirus isolates from *Clethrionomys glareolus* and *Apodemus flavicollis*. *Acta Virol* 1982;25:101-7.
7. Sunil-Chandra NP, Efstathiou S, Arno J, Nash AA. Virological and pathological features of mice infected with murine gamma-herpesvirus 68. *J Gen Virol* 1992;73:2347-56.
8. Sunil-Chandra NP, Efstathiou S, Nash AA. Interactions of murine gammaherpesvirus 68 with B and T cell lines. *Virology* 1993;193:825-33.
9. Usherwood EJ, Stewart JP, Nash AA. Characterization of tumor cell lines derived from murine gammaherpesvirus-68-infected mice. *J Virol* 1996;70:6516-8.
10. Rooney CM, Rickinson AB, Moss DJ, Lenoir GM, Epstein MA. Paired Epstein-Barr virus-carrying lymphoma and lymphoblastoid cell lines from Burkitt's lymphoma patients: comparative sensitivity to non-specific and to allo-specific cytotoxic responses in vitro. *Int J Cancer* 1984;34:339-48.
11. Efstathiou S, Ho YM, Minson AC. Cloning and molecular characterization of the murine herpesvirus 68 genome. *J Gen Virol* 1990;71:1355-64.
12. Efstathiou S, Ho YM, Hall S, Styles CJ, Scott SD, Gompels UA. Murine herpesvirus 68 is genetically related to the gammaherpesviruses Epstein-Barr virus and herpesvirus saimiri. *J Gen Virol* 1990;71:1365-72.
13. Mackett M, Stewart JP, Pepper S, de V, Chee M, Efstathiou S, Nash AA, *et al.* Genetic content and preliminary transcriptional analysis of a representative region of murine gamma-herpesvirus 68. *J Gen Virol* 1997;78:1425-33.
14. Virgin HW, Latreille P, Wamsley P, Hallsworth K, Weck KE, Dal Canto AJ, *et al.* Complete sequence and genomic analysis of murine gammaherpesvirus 68. *J Virol* 1997;71: 5894-904.
15. Bowden RJ, Simas JP, Davis AJ, Efstathiou S. Murine gammaherpesvirus 68 encodes tRNA-like sequences which are expressed during latency. *J Gen Virol* 1997;78:1675-87.
16. Simas JP, Bowden RJ, Paige V, Efstathiou S. Four tRNA-like sequences and a serpin homologue encoded by murine gammaherpesvirus 68 are dispensable for lytic replication in vitro and latency in vivo. *J Gen Virol* 1998;79:149-53.
17. Stewart JP, Usherwood EJ, Ross A, Dyson H, Nash T. Lung epithelial cells are a major site of murine gammaherpesvirus persistence. *J Exp Med* 1998;187:1941-51.
18. Simas JP, Swann D, Bowden R, Efstathiou S. Analysis of murine gammaherpesvirus-68 transcription during lytic and latent infection. *J Gen Virol* 1999;80:75-82.
19. Sunil-Chandra NP. Studies on the pathogenesis of a murine gammaherpesvirus (MHV-68) [PhD]. University of Cambridge, 1991.
20. Rajcani J, Blaskovic D, Svobodova J, Ciampor F, Huckova D, Stanekova D. Pathogenesis of acute and persistent murine herpesvirus infection in mice. *Acta Virol* 1985;29:5160.
21. Blaskovic D, Stanekova D, Rajcani J. Experimental pathogenesis of murine herpesvirus in newborn mice. *Acta Virol* 1984;28:225-31.
22. Sunil-Chandra NP, Efstathiou S, Nash AA. Murine gammaherpesvirus 68 establishes a latent infection in mouse B lymphocytes in vivo. *J Gen Virol* 1992;73:3275-9.
23. Usherwood EJ, Stewart JP, Robertson K, Allen DJ, Nash AA. Absence of splenic latency in murine gammaherpesvirus 68-infected B cell-deficient mice. *J Gen Virol* 1996;77:2819-25.
24. Weck KE, Barkon ML, Yoo LI, Speck SR, Virgin HI. Mature B cells are required for acute splenic infection, but not for establishment of latency, by murine gammaherpesvirus 68. *J Virol* 1996;70:6775-80.
25. Weck KE, Kim SS, Virgin HW, Speck SH. Macrophages are the major reservoir of latent murine gammaherpesvirus 68 in peritoneal cells. *J Virol* 1999;73:3273-88.
26. Tripp RA, Hamilton-Easton AM, Cardin RD, Nguyen P, Behm FG, Woodland DL, *et al.* Pathogenesis of an infectious mononucleosis-like disease induced by a murine gamma-herpesvirus: role for a viral superantigen? *J Exp Med* 1997;185:1641-50.
27. Lung ML, Lam WK, So SY, Lam WP, Chan KH, Ng MH. Evidence that the respiratory tract is a major reservoir for Epstein-Barr virus. *Lancet* 1985;1:889-92.
28. Egan JJ, Stewart JP, Hasleton PS, Arrand JR, Carroll KB, Woodcock AA. Epstein-Barr virus replication within pulmonary epithelial cells in cryptogenic fibrosing alveolitis. *Thorax* 1995;50:1234-9.
29. Stewart JP, Egan JJ, Ross AJ, Kelly BG, Lok SS, Hasleton PS, *et al.* The detection of Epstein-Barr virus DNA in lung tissue from patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1999. In Press.
30. Di Alberti L, Piattelli A, Artese L, Favia G, Patel S, Saunders N, *et al.* Human herpesvirus 8 variants in sarcoid tissues. *Lancet* 1997;350:1655-61.
31. Sunil-Chandra NP, Amo J, Fazakerley J, Nash AA. Lymphoproliferative disease in mice infected with murine gammaherpesvirus 68. *Am J Pathol* 1994;145:818-26.
32. Pepper SD, Stewart JP, Arrand JR, Mackett M. Murine gammaherpesvirus-68 encodes homologues of thymidine kinase and glycoprotein H: sequence, expression, and characterization of pyrimidine kinase activity. *Virology* 1996;219:475-9.
33. Stewart JP, Janjua NJ, Sunil-Chandra NP, Nash AA, Arrand JR. Characterization of murine gammaherpesvirus 68 glycoprotein B (gB) homolog: similarity to EpsteinBarr virus gB (gp 1 10). *J Virol* 1994;68:6496-504.
34. Stewart JP, Janjua NJ, Pepper SD, Bennion G, Mackett M, Allen T, *et al.* Identification and characterization of murine gammaherpesvirus 68 gpl50: a virion membrane glycoprotein. *J Virol* 1996;70:3528-35.
35. Virgin HI, Presti RM, Li XY, Liu C, Speck SH. Three distinct regions of the murine gammaherpesvirus 68 genome are transcriptionally active in latently infected mice. *J Virol* 1999;73:2321-32.
36. Husain SM, Usherwood EJ, Dyson H, Coleclough C, Cappola M, Woodland DL, *et al.* Murine gammaherpesvirus M2 gene is latency associated and a target for CD8+ T lymphocytes. *Proc Natl Acad Sci USA* 1999. In Press.
37. Stevenson PG, Doherty PC. Kinetic analysis of the specific host response to a murine gammaherpesvirus. *J Virol* 1998;72:943-9.
38. Ehtisham S, Sunil-Chandra NP, Nash AA. Pathogenesis of murine gammaherpesvirus infection in mice deficient in CD4 and CD8 T cells. *J Virol* 1993;67:5247-52.
39. Cardin RD, Brooks JW, Sarawar SR, Doherty PC. Progressive loss of CD8+ T cell-mediated control of a gamma-herpesvirus in the absence of CD4+ T cells. *J Exp Med* 1996;184:863-71.

40. Stevenson PG, Cardin RC, Christensen JP, Doherty PC. Immunological control of a murine gammaherpesvirus independent of CD8+ T cells. *J Gen Virol* 1999;80:477-83.
41. Usherwood EJ, Ross AJ, Allen DJ, Nash AA. Murine gammaherpesvirus-induced splenomegaly: a critical role for CD4 T cells. *J Gen Virol* 1996;77:627-30.
42. Sarawar SR, Cardin RD, Brooks JW, Mehrpooya M, Tripp RA, Doherty PC. Cytokine production in the immune response to murine gammaherpesvirus 68. *J Virol* 1996;70:3264-8.
43. Sarawar SR, Cardin RD, Brooks JW, Mehrpooya M, Hamilton-Easton AM, Mo XY, *et al.* Gamma interferon is not essential for recovery from acute infection with murine gammaherpesvirus 68. *J Virol* 1997;71:3916-21.
44. Sarawar SR, Brooks JW, Cardin RD, Mehrpooya M, Doherty PC. Pathogenesis of murine gammaherpesvirus-68 infection in interleukin-6-deficient mice. *Virology* 1998;249:359-66.
45. Stevenson PG, Doherty PC. Non-antigen-specific B-cell activation following murine gammaherpesvirus infection is CD4 independent in vitro but CD4 dependent in vivo. *J Virol* 1999;73:1075-9.
46. Kulkami AB, Holmes KL, Fredrickson TN, Hartley JW, Morse HC. Characteristics of a murine gammaherpesvirus infection immunocompromised mice. *In Vivo* 1997;11:281-91.
47. Dutia BM, Clarke CJ, Allen DJ, Nash AA. Pathological changes in the spleens of gamma interferon receptor-deficient mice infected with murine gammaherpesvirus: a role for CD8 T cells. *J Virol* 1997;71:4278-83.
48. Rooney CM, Rowe M, Wallace LE, Rickinson AB. Epstein-Barr virus-positive Burkitt's lymphoma cells not recognized by virus-specific T-cell surveillance. *Nature* 1985;317:629-31.
49. Weck KE, Dal Canto AJ, Gould JD, O'Guin AK, Roth KA, Saffitz JE, *et al.* Murine gamma-herpesvirus 68 causes severe large-vessel arteritis in mice lacking interferon gamma responsiveness: a new model for virus-induced vascular disease. *Nat Med* 1997;3:1346-53.
50. Sunil-Chandra NP, Efstathiou S, Nash AA. The effect of acyclovir on the acute and latent murine gammaherpesvirus-68 infection of mice. *Antivir Chem Chemother* 1994;5:290-6.
51. Smee DF, Burger RA, Warren RP, Bailey KW, Sidwell RW. An immunosuppressed mouse model of lethal murine gammaherpesvirus 68 infection for studying potential treatment of Epstein-Barr virus infection in man. *Antivir Chem Chemother* 1997;8:573-81.
52. Neyts J, De Clercq E. In vitro and in vivo inhibition of murine gamma herpesvirus 68 replication by selected antiviral agents. *Antimicrob Agents Chemother* 1998;42:170-2.
53. Stewart JP, Micali N, Usherwood EJ, Bonina L, Nash AA. Murine gamma-herpesvirus 68 glycoprotein 150 protects against virus-induced mononucleosis: a model system for gamma-herpesvirus vaccination. *Vaccine* 1999;17:152-7.