Chapter 14
Murine Gammaherpesvirus 68: A Small Animal Model for Gammaherpesvirus-Associated Diseases

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Abstract Murine gammaherpesvirus 68 (MHV68) is a naturally occurring pathogen of murid rodents that is genetically related to the human gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV). Viral, immunologic, and disease parameters following experimental infection of laboratory mice with MHV68 closely resemble what occurs during primary EBV infection of humans, which suggests that MHV68 infection of mice offers a small animal model to study in general the pathogenesis of gammaherpesvirus infections. Diseases elicited by MHV68 infection include lymphoproliferative diseases, idiopathic pulmonary fibrosis, and autoimmune diseases, ailments also associated with EBV infection of humans. Furthermore, MHV68 infection also is linked to the development of vasculitis, encephalomyelitis, and other disorders that resemble pathologies with viral and nonviral etiologies in humans. This review aims to provide an overview of MHV68-associated diseases in infected mice that may provide a model for understanding basic mechanisms by which similar diseases in humans occur and can be treated.

Keywords Animal model • Murine gammaherpesvirus 68 • Epstein-Barr virus • Kaposi sarcoma-associated herpesvirus

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14.1 Introduction

Murine gammaherpesvirus 68 (MHV68) is a naturally occurring virus that was originally isolated from Slovakian bank voles and is endemic in European wood mice [1–3]. MHV68 is genetically related to the human gammaherpesviruses (GHVs), Epstein-Barr virus (EBV), and Kaposi sarcoma-associated herpesvirus (KSHV), possessing a genome that is collinear with those of EBV and KSHV and contains large blocks of conserved genes with interspersed unique genes as well [1, 4, 5]. Following intranasal infection of mice, MHV68 undergoes acute infection in the lungs and nasal epithelium and establishes latency, a nonproductive, quiescent infection characterized by minimal viral gene expression and maintenance of the viral genome, in cells of the spleen and blood [6–9]. Productive replication mainly involves epithelial and mononuclear cells in the lungs, with acute infection resolving by approximately 2 weeks post-infection [10]. Acute replication precedes and is thought to be necessary for lifelong latent infection in lymphoid tissues, where B cells serve as the major latent reservoir for MHV68 in the spleen [6]. Peritoneal macrophages and lung epithelial cells also harbor latent MHV68 genomes [10, 11].

MHV68 infection of mice results in a variety of pathologies that resemble EBV-associated diseases and other human disorders. Mice chronically infected with MHV68 develop a marked splenomegaly and lymphoproliferative diseases (LPDs), similar to what is observed in patients infected with EBV [12–14]. MHV68 infection induces multi-organ fibrosis and vasculitis in interferon gamma receptor knockout (IFNγR-/-) mice [15–17]. In other disease models, MHV68 infection promotes systemic inflammation, exacerbates autoimmune encephalomyelitis, and influences development of other pathologies [18, 19]. Here, we will discuss MHV68-related diseases and the potential value of this small animal model for the study of similar diseases associated with infections by human GHVs.

14.2 MHV68-Associated Diseases

14.2.1 MHV68-Associated Lymphoproliferative Diseases

Infection of laboratory mice with MHV68 leads to a variety of pathological changes that mirror EBV-associated LPDs and other malignancies. Following intranasal infection of wild-type mice with MHV68, acute infection in the lung develops and is subsequently cleared, followed by the establishment of latency in the spleen [20, 21]. Latency establishment is accompanied by splenomegaly, which is characterized by a two- to threefold increase in the number of spleen cells, with the largest increase occurring in the CD8+ T cell population [13]. Polyclonal B-cell activation and autoantibody production also occur [13]. This is similar to what occurs during EBV-induced infectious mononucleosis in humans [22, 23]. Development of splenomegaly in MHV68 infection requires CD4+ T cells and organized secondary

lymphoid tissue [13, 24, 25]. CD25-mediated IL-2 signaling also is necessary for the CD8+ T cell mononucleosis that occurs [26].

BALB/c mice chronically infected with MHV68 develop LPDs, including high-grade lymphomas that resemble centroblastic or plasmablastic non-Hodgkin lymphomas seen in humans [12]. MHV68-associated lymphomas primarily occur in older mice (0.75–3 years of age), and lymphoma incidence is greatly increased when infected mice are treated with the immunosuppressive drug cyclosporin A [12]. Since cyclosporin A functions chiefly through inhibition of T cell function, this finding strongly suggests that T cells are important for limiting tumor growth in MHV68-infected mice. Indeed, adoptive transfer of CD4+ T cells from infected mice promotes regression of lymphomas that developed following subcutaneous injection of an MHV68-positive B-cell lymphoma line, S11, isolated from a tumor-bearing BALB/c mouse [27]. Although MHV68 does not appear to transform primary murine B cells in culture, murine fetal liver-derived B cells are transformed by MHV68 into plasmablast-like B cells in vitro [28]. Similar to S11 cells, when these plasmablast-like B cells are injected into immunodeficient mice, the transformed B cells induce lymphomas that can be controlled by both CD4+ and CD8+ T cells [29]. Together, these findings illustrate (i) that MHV68 infection can cause lymphomas and (ii) that T cells are important for controlling infection-associated lymphomas.

MHV68 infection of BALB/c_2 microglobulin (B2M)-deficient mice (BALB B2M-/-) also results in B-cell lymphoma and an atypical lymphoid hyperplasia (ALH) [14]. ALH pathologically is differentially regulated by MHV68 genes and resembles posttransplant lymphoproliferative disease observed in some EBV-infected individuals that are immune suppressed for solid organ transplants [30, 31]. B2M is a critical component of the major histocompatibility I (MHCI) complex, a cell surface receptor necessary for CD8+ T cells to engage target cells [32]. This further illustrates the importance of T cells in preventing MHV68-associated LPDs.

Lymphomatoid granulomatosis (LYG) is a rare systemic angiodestructive LPD caused by the combination of EBV infection and immunosuppression [33, 34]. LYG mostly affects the lungs and is recently characterized as B-cell lymphomas with prominent pulmonary involvement [33]. MHV68-infected IFNγR-/- mice also develop pulmonary B-cell lymphomas which closely mimic EBV-associated LYG in human [35].

Nevertheless, there are differences between EBV-associated LPDs in humans and MHV68-associated LPDs in mice. For example, CD8+ T cell lymphocytosis associated with EBV-induced mononucleosis is predominantly an outgrowth of T cells responding to viral lytic epitopes [36, 37]. In contrast MHV68-induced mononucleosis in C57BL/6 mice represents expansion of CD8+ T cells that encode a Vβ4 T cell receptor that is not reactive to viral epitopes and appears to be stimulated by latently infected B cells [38, 39]. However, the striking pathological similarities between EBV-associated LPDs and the corresponding syndrome in MHV68-infected mice highlight MHV68 as a valuable small animal model for studying fundamental issues in gammaherpesvirus-associated LPD pathogenesis in a natural host.

14.2.2 MHV68-Associated Fibrosis

Several reports associate EBV infection with idiopathic pulmonary fibrosis (IPF), a chronic, progressive, fibrotic lung disorder of unknown etiology that is a risk factor for lung cancer development [40–43]. Although EBV is frequently detected in lung tissues of patients with IPF, an etiologic role for EBV in IPF is not established REF. Interestingly, MHV68 infection of IFNγR-/- mice leads to multi-organ fibrosis, which occurs in the lung, spleen, mediastinal lymph nodes, and liver of these mice [15, 16, 44, 45]. Lung fibrosis in MHV68-infected IFNγR-/- mice shares similar pathology to IPF in humans [45]. Mechanistic studies show that both viral and cellular factors are involved in MHV68-induced fibrosis in IFNγR-/- mice. Persistent MHV68 lytic replication apparently is essential for induction or exacerbation of IPF, because severe fibrosis is ameliorated in MHV68-infected IFNγR-/- mice that receive antiviral treatment and in IFNγR-/- mice infected with a reactivation-defective MHV68 mutant that fails to express v-cyclin [46]. Moreover, MHV68 superantigen-like M1 protein and activated Vβ4+ CD8+ T cells, which are driven to expand by M1, also are required for MHV68-induced inflammation and fibrosis in IFN-γR-/- mice [47, 48]. Additionally, inhibition of NF-κB signaling reduces virus persistence and pulmonary fibrosis in MHV68-infected IFNγR-/- mice, indicating that NF-κB signaling also is important for MHV68-induced pulmonary fibrosis [49]. Thus, MHV68 infection of IFNγR-/- mice could be used to model the association of gammaherpesvirus infection with IPF and define underlying molecular mechanisms of disease.

MHV68 infection of bleomycin-resistant BALB/c mice has also been used to study the association between GHV infection and IPF. Bleomycin-induced fibrosis is widely used experimental model for lung fibrosis occurring during chemotherapy [50]. BALB/c mice are inherently resistant to lung fibrosis due to bleomycin treatment and do not develop pulmonary fibrosis when infected with MHV68. However, when BALB/c mice are simultaneously infected with MHV68 and treated with bleomycin, lung fibrosis occurs [51], indicating that MHV68 functions as a cofactor in bleomycin-induced fibrosis. Another study demonstrated that TLR9 signaling protects against MHV68-induced exacerbation of lung fibrosis induced by bleomycin in BALB/c mice [52]. These findings support the role of GHV infection in human IPD, and future development of the MHV68/bleomycin model should further explore mechanisms by which GHV infection functions as a cofactor in the pathogenesis of pulmonary fibrosis.

Finally, MHV68 infection of IFNγ deficient (IFNγ-/-) mice on the BALB/c genetic background results in acute lethal pneumonia that is dependent on MHV68-encoded v-cyclin and v-bcl-2 [53]. However, whether MHV68-induced pneumonia in IFNγ-/- mice is pathologically similar to EBV-associated pneumonia, which mainly occurs in children and transplant patients, is not yet clear [54–57].

In addition to lung fibrosis, MHV68 infection of IFNγR-/- mice also induces fibrosis in the spleen. The prominent feature of splenic pathology in infected IFNγR-/- mice is a loss of B cells and CD4+ and CD8+ T cells, which correlates with

significant changes in cytokines and chemokines in spleens. In contrast, a dramatic increase in T and B lymphocytes in peripheral blood occurs [15, 44]. CD8+ T cells are the major mediators of splenic damage, since depletion of CD8+ T cells completely reverses the pathological and histological changes in spleens of these mice. However, although removal of CD4+ T cells reverses the weight loss and reduces the number of infective centers, some pathological changes are still observed in CD4+ T cell-depleted mice. This suggests that CD4+ T cells are not the dominant mediators but still play an important role in splenic fibrosis [44].

Furthermore, MHV68 infection of IFNγR-/- mice leads to enhanced production of Th2 cytokines IL-5, IL-13, and IL-21 and increased expression of CCR4 in the spleens of infected mice [16]. This drives alternative activation of macrophages to produce arginase 1 (ARG1) and found in inflammatory zone 1 (FIZZ1)/resistin-like molecule-α (RELMα) to promote fibrotic disease in the spleen [16, 58]. Though EBV infection is not directly linked to fibrotic disease of the spleen in humans, the data from MHV68 infections suggest a role for GHV infection in such diseases. This model may therefore hold future relevance for understanding how viruses influence splenic fibrosis in general.

14.2.3 MHV68 Impact on Autoimmune Diseases

Multiple sclerosis (MS) is an autoimmune disorder in which the immune system attacks the central nervous system (CNS), damaging the myelin sheath of nerve cells in the brain and spinal cord. EBV is etiologically linked to MS [59–61]; however mechanisms by which EBV influences MS pathogenesis are not known. In mice induction of inflammatory immune responses in the brain triggers an MS-like syndrome known as experimental autoimmune encephalomyelitis (EAE) [62, 63]. Because MHV68 replicates in the mouse brain, infecting microglia and astrocytes [64, 65], and globally influences immune activation in infected animals [66], the impact of MHV68 infection on EAE pathogenesis was evaluated. Latent MHV68 infection enhances EAE pathogenesis and central nervous system pathology in a manner reminiscent of human MS [19, 67]. This observation demonstrates that GHV infection can influence the course of disease in CNS autoimmune disorders and highlights the potential of these small animal models in facilitating an understanding of mechanisms by which EBV influences MS.

EBV infection also is linked to development of lupus in humans, an autoimmune disease in which healthy tissues are attacked by the individual's immune system, leading to swelling and damage of various tissues of the body. In contrast to EAE models, MHV68 infection protects, rather than exacerbates, lupus-prone mice from the development and progression of autoimmunity [68]. Together, these findings demonstrate that GHV infection influences the course of CNS autoimmune disease. However, the data also demonstrate that pathogenesis is likely a multifactorial process in which GHV infections may have pleiotropic impacts on disease progression.

The impact of MHV68 infection in other mouse models of autoimmune disease also has been evaluated. For instance, IL10$^{-/-}$ mice are prone to developing inflammatory bowel disease (IBD), and infection with MHV68 promotes more rapid and severe disease in these mice [69]. This finding is similar to the observation that EBV infection correlates with disease severity in some IBD patients [70–74]. In contrast, MHV68 infection of nonobese diabetic (NOD) mice, a mouse model for evaluating type I diabetes (T1D), significantly delays diabetes onset [75], which supports the hypothesis that viruses are potential regulators of T1D [76–78]. Furthermore, transgenic mice expressing MHV68 chemokine decoy receptor M3 in beta cells are remarkably resistant to diabetes induced by multiple low doses of streptozotocin [79]. This suggests the importance of specific viral factors in regulating T1D. Together, these data highlight the manner in which MHV68 studies could be employed to define roles for GHVs in intestinal diseases and diabetes.

14.2.4 MHV68-Related Vascular and Ductal Disorders

In addition to lymphoma development and fibrosis, MHV68 causes severe large-vessel arteritis associated with lipid accumulation in the vessel wall and luminal thrombosis in IFNγR-/- mice. Lesions that develop are similar to those seen during the acute inflammatory phase of Takayasu arteritis, the nongranulomatous variant of temporal arteritis and Kawasaki diseases [17], suggesting possible GHV etiologies in these pathologies and demonstrating the utility of MHV68 infection of mice in dissecting GHV roles in human vasculitis. Furthermore, MHV68 induces chronic inflammation of intrahepatic bile ducts in infected IFNγR-/- mice, which is pathologically similar to the human fibrotic liver disorder primary sclerosing cholangitis [80]. Additionally, MHV68 reactivation from latency induces neointimal lesions in pulmonary arteries of S100A4/Mts1-overexpressing mice. These lesions are associated with elevated neutrophil elastase, which is produced by pulmonary artery smooth muscle cells and linked to experimental and clinical pulmonary vascular disease [81, 82]. Finally, MHV68 infection in mice also induces phenotypes that mimic rare diseases such as systemic lymphocytosis following gastric instillation and fatigue [83, 84]. MHV68 may therefore provide a useful model for the study of fatigue and other physiologic and behavioral perturbations that occur during acute and chronic infection with gammaherpesviruses.

14.3 Remarks and Perspectives

Human gammaherpesviruses are exquisitely species restricted, which limits possible approaches for defining precise mechanisms by which these viruses cause disease. The beauty of small animal models of viral pathogenesis is that they enable evaluations of both viral and host determinants of disease in experimentally

controlled settings. In contrast, analogous studies of GHV infections in humans would require the presence of naturally occurring mutations in either virus or host, paired with the ability to identify individuals and viruses that possess such genetic variants. Even then, the studies would be necessarily associative, observational, and potentially influenced by numerous outside variables due to environment, lifestyle, additional genetic variations, age, coinfections, etc. The use of humanized mice, immunodeficient animals in which the immune system is reconstituted by human stem cells, allows an experimental system for evaluating certain aspects of GHV infection in human cells. But again the system is still genetically limited and may not faithfully recapitulate natural cellular development and cell-cell interactions, and not all tissues in the reconstituted mouse are of human origin. Humanized mice are also very expensive. Hence, the capacity to study a genetically related pathogen (MHV68) in a natural host (rodents) offers a powerful tool for understanding virus-host interactions in GHV infection-associated diseases. Here, infections of inbred mice with MHV68 provide a simplified and standardized analysis of immune responses against the virus and eliminate many potential experimental variables. Moreover, the ease of genetically manipulating both virus and host further highlights the tractability of the MHV68 system. Indeed, genetically modified mice and viruses enable many of disease models described above.

However, this is not to say that infection of mice with MHV68 is identical to human infections with EBV or KSHV. Clearly mice are not humans, and it is naïve to think that all aspects of the host response will be identical in two quite divergent species. Further, while genetic diversity is an experimental problem, genetic polymorphisms from human to human and population to population undoubtedly influence the pathogenesis and outcome of infection by GHVs.

Along these same lines, MHV68 is not EBV or KSHV. While all GHVs (and herpesviruses in general) possess blocks of conserved genes, each virus also encodes unique proteins that are not shared with their GHV relatives. These genes maintain no vestige of sequence homology and may have developed through convergent evolution to satisfy unique requirements of the virus-host relationship. It is however interesting to note that the products of these divergent genes likely perform conserved functions, for instance, both LMP2a of EBV and M2 of MHV68 manipulate B-cell survival and differentiation [85]. If these unique genes are under selection from the host, it is equally possible that they have simply diverged over millennia at a rate that made them nonhomologous at the sequence level by modern informatics techniques while maintaining critical functions. Perhaps the key take-home points are these: MHV68 provides a highly tractable experimental system for understanding how GHVs influence disease in a variety of experimental models. Though obvious differences exist between human and mouse infections, the data produced in the mouse models are real and may offer invaluable insights into factors that influence similar diseases in humans. As such, MHV68 infection of mice can serve as powerful tool in the arsenal for illuminating previously unappreciated factors and cofactors that influence human disease and allow for preclinical testing of novel hypotheses for treating related diseases.

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