MURINE HEPATITIS VIRUS STRAIN 1 AS A MODEL FOR SEVERE ACUTE RESPIRATORY DISTRESS SYNDROME (SARS)

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1. INTRODUCTION

Severe acute respiratory syndrome (SARS) is a novel infectious disorder that was first diagnosed in China in November 2002.[1,2] SARS was documented in approximately 8,000 persons globally with more than 700 deaths. In Canada, there were 375 probable and suspect cases between March and July 2003 with 44 deaths, reflecting a mortality rate of 11%. Spread of SARS was shown to be by airborne droplets and results in acute pulmonary inflammation and epithelial damage.[3] It has now been determined that a novel coronavirus, SARS-CoV, is the etiologic agent in SARS. Based on phylogenetic sequence analysis, it best fits within group 2 coronaviruses, which include the mouse hepatitis viruses (MHV).[4,5]

As for most infections, SARS varies considerably in terms of its clinical severity. This variation is almost certainly due to population-based diversity in the genes controlling the immune response. Clearance of mouse hepatitis virus coincides with a robust innate immune response, including increased numbers of CD8 T cells. Disease and death do not correlate with high viral titers, and it has been suggested that disease reflects alteration in host innate immune response. Furthermore, host production and response to type 1 interferons (IFN) is a key determinant of outcome in MHV-infected mice.[6] However, IFNs and other cytokines regulate in a coordinate manner both inflammation and the Th1/Th2 character of the specific immune response. An imbalance in timing and proportions of cellular responses to inflammatory cytokines after viral infection can lead to chronic disease or death. Although a number of models for SARS have been proposed including SARS-CoV infection of mice, of cats and ferrets, and SARS-CoV infection of non-human primates, none of the models produce lung pathology similar to that seen in

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humans and serve only as models where agents including neutralizing antibodies, putative vaccines, or anti-virals can be studied for effect on viral replication.[7,8]

MHV-1 was first isolated in 1950, and mice infected by MHV-1 had massive hepatic necrosis on autopsy. On further analysis, it was realized that MHV-1 infected mice were co-infected with *Eperythrozoon coccoides*. When mice were infected with MHV-1 devoid of this bacterium, only mild hepatitis was seen and all mice survived. As described below, the MHV-1 mouse model established in our laboratory offers the potential to provide insights into the pathogenesis of MHV-induced lung injury and the contribution of both the virus and host immune response.

2. MATERIALS AND METHODS

Mice: Female Balb/cJ, A/J, and C3H mice, 6–8 weeks of age, were purchased from Jackson Laboratories and housed in the animal facility of the Toronto General Research and were fed with standard laboratory chow diet and water *ad libitum*.

Virus: MHV-1, MHV-A59, MHV-JHM, MHV-S, and MHV-3 was originally obtained from American Type Culture Collection (ATCC) and plaque purified on monolayers of DBT cells and titered on L2 cells using a standard plaque assay.[9]

Tissue processing: Lungs, spleens, livers, kidneys, small intestines, hearts, and brains were harvested from mice and samples snap frozen and stored in a -80^{o}C freezer or fixed with 10% formalin for further analysis. For detection of fgl2 and fibrin, a standard immunohistochemical system was employed as previously described.

Cytokine assays: Serum cytokine levels were assayed using commercial cytometric bead array kits (BD Biosciences) for IL-6, IL-10, IL-12p70, IFN-γ, TNF-α, MCP-1. Samples were analyzed in triplicate using a BD FACS Calibur flow cytometer.

3. RESULTS

Balb/cJ mice infected with 10^5 pfu of MHV-1 intranasally developed severe pulmonary disease characterized by congestion, pulmonary infiltrates, hyaline membranes, and hemorrhage. In addition to diffuse pulmonary infiltrates, focal deposition of fibrin was seen around small arterial blood vessels and in alveolar spaces with entrapment of platelets. Changes were noted as early as 3 days p.i. and progressed to day 28. Clinically these mice became lethargic, with rapid respiration, but all of the mice survived and pulmonary pathology resolved within 21 days of infection. Balb/cJ mice infected intranasally with MHV-JHM developed neither liver or lung pathology. Although MHV-3 and MHV-A59 produced pulmonary lesions, these were milder than those generated by MHV-1 and did not have the characteristics of lesions caused by SARS. MHV-A59 and MHV-3 infected mice all developed severe hepatic necrosis and died of liver failure by day 10 and thus these strains of MHV do not represent relevant models of SARS (Table 1).

Table 1. Effect of different strains of MHV on lung pathology of Balb/cJ mice.

	MHV strain				
Features of SARS	MHV-1	MHV-3	MHV A59	MHV- S	MHV JHM
Congestion	Marked		X		
Edema			Few patches		
Hyaline membrane	X				
Interstitial thickening	X	X	X	X	Minimal
Airways	Inflammation, bronchopneumonia				
Pattern	Hemorrhage particularly in anterior portion		Changes in subpleural regions, perhaps giant		

These data suggest that MHV-1 induces a pathology most similar to human SARS. Therefore, we infected 3 inbred strains of mice (C57Bl/6J, C3H/HeJ, and A/J mice) that have previously been known to show varying degrees of susceptibility to other strains of MHV. Although C57Bl/6J developed acute pulmonary disease, these mice all survived and the pulmonary lesions resolved by day 21. C3H/HeJ mice showed an intermediate pattern of resistance/susceptibility with 40% of mice dying by day 28. Surviving C3H mice developed pulmonary fibrosis and bronchial hyperplasia (Figure 2). A/J mice all died within 7–10 days post–MHV-1 infection of severe pulmonary disease. Lungs showed 100% consolidated pneumonitis with hyaline membranes, fibrin deposition, and lymphocytic and macrophage infiltration (Figure 1A). We examined lung tissue from both susceptible A/J and resistant C57Bl/6J mice for presence of fgl2 mRNA transcripts by real-time PCR, protein, and fibrin, as fgl2 is known to cause thrombosis. Shown in Figure 1B, fgl2 protein was expressed by inflammatory cells and type 1 pneumocytes in juxtaposition with deposits of fibrin. Lungs from Balb/cJ and C57Bl/6 mice had neither fgl2 or fibrin deposits. Electron micrographs of lung showed virions mostly localized to type 1 pneumocytes and pulmonary macrophages (data not shown). By plaque assay, virus was detected in the lung by 12 hours, reaching maximal levels by 48–72 hours and in A/J mice persisted at high levels until death of animals. Virus was also detected in the lungs of C57Bl/6J mice by 12 hours p.i., reaching maximal levels by day 4 but disappearing by days 7–10 p.i.

Serum was collected from A/J and C57Bl/6J mice pre and post MHV-1 infection and cytokines measured (Table 2). Gene expression levels for the different IFNαs and IFN-ß were assessed in lung tissues from MHV-1 infected C57Bl/6Jand A/J mice, using quantitative real-time PCR. In contrast to the late (36 hr postinfection) and low levels of induction of IFN-αs and IFN-ß in the A/J mice, we observed a robust and sustained IFN-α and IFN-ß gene induction by 12 hr postinfection in the C57BL/6 mice (data not

shown). The data suggest a correlation between the kinetics and extent of an IFN response and disease severity.

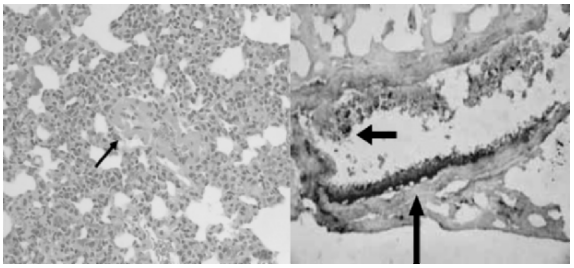

Figure 1. Left: SARS-like lung pathology in A/J mice infected with coronavirus MHV-1, day 7, postinfection. Severe interstitial pneumonitis with hyaline membranes (arrow). Most bronchi remain widely open but the alveolar spaces are completely consolidated. Right: Co-localization of fgl2 and fibrin detected by double immunochemistry staining. Widespread fibrin deposition near fgl2 expression, especially in microvasculature (arrows) of the lung.

Table 2. Serum cytokine profiles in MHV-1 infected mice (pg/mL).

	Day 0 p.i.		Day 6 p.i.	
Cytokines	AJ	C57BL6/J	AJ	C57BL6/J
IL-10	26.7±3	24.6±4	164±22	38±12
IL-6	53.8±4.9	48.6±5	546±23	215±20
IL-12p70	306±30.6	298±29	642.5±36	449.3±21
IFN-γ	14.7±1.4	13.9±2	555±62	236±34
TNF-α	25.2±2.5	18±2	163±22	68±12
MCP-1	166±16	159±16	7400±400	225±40

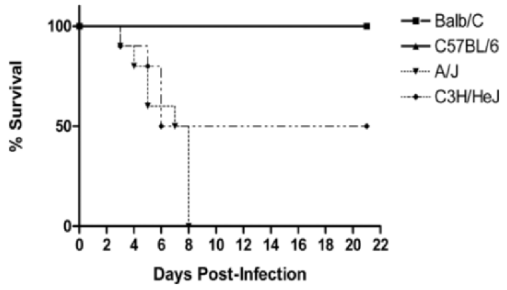

Figure 2. Mortality in MHV-1 infected (1 x 10^5 pfu) intranasally (n-10 per group).

4. DISCUSSION

Existing models for SARS in rodents and non-human primates fail to produce lung pathology or mortality similar to that seen in humans. Thus, these models only serve to assess the effects of agents including neutralizing antibodies, putative vaccines, or anti-virals on viral replication. The MHV-1 model described in this report produces a clinical syndrome in mice that serves as a model for SARS. First, we found that MHV-1 produces strain-dependent disease. Interestingly, A/J mice usually resistant to MHV-3 and MHV-A59 infection all died after intranasal infection with MHV-1. In contrast, although Balb/cJ and C57Bl/6 mice developed pulmonary disease, these animals cleared virus by day 14 and survived. C3H mice developed an intermediate pattern of susceptibility with a 40% mortality. Viral titers were higher in susceptible A/J mice and serum cytokines and chemokines were markedly elevated in these mice in comparison with resistant animals. These findings are consistent with the elevated levels of IFNγ, TNFα, IL-12p70, and IL-8 (CXCL8) detected in sera from SARS patients.[10] These elevated cytokines and chemokines could contribute to the immunopathology of SARS. The fact that corticosteroids ameliorated disease in some SARS patients is consistent with this.

We previously reported that MHV-3 induces fgl2, an inflammatory immune coagulant, which results in fibrin deposition and hepatic necrosis. In the MHV-1 model of SARS, both fgl2 mRNA transcripts and protein were also seen in association with deposits of fibrin in diseased lungs from A/J mice suggesting that this inflammatory mediator may be contributing to the pathogenesis of SARS as well. These results are compatible with what has been reported in humans with SARS CoV.

Susceptible animals failed to generate a robust type 1 interferon response, which, in addition to their anti-viral effects are known to inhibit inflammatory cytokines. IFN alfacon treatment of SARS patients accelerated resolution of inflammation, possibly contributing to increased survival consistent with the above observations. Thus collectively, these data support the concept that the pathogenesis of SARS reflects an altered innate immune response with marked inflammation. The molecular mechanism for these findings is not presently known.

This model offers the potential to conduct additional studies that will provide insights into the pathogenesis of coronavirus-induced lung injury and the contribution of both the virus and host immune response. We anticipate that data generated from these studies will provide novel insights into the pathogenesis of this serious human disease and provide avenues for therapy.

5. ACKNOWLEDGMENTS

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