Published in final edited form as:

J Hepatol. 2018 April ; 68(4): 733–743. doi:10.1016/j.jhep.2017.11.010.

Macrophage scavenger receptor 1 contributes to pathogenesis of fulminant hepatitis via neutrophil-mediated complement activation

Yuan Tang¹, Huifang Li¹, Junru Li¹, Yunzhi Liu¹, Yanli Li¹, Jing Zhou¹, Jia Zhou¹, Xiao Lu¹, Wei Zhao², Jinlin Hou³, Xiang-Yang Wang⁴, Zhengliang Chen^{1,5,*}, and Daming Zuo^{1,5,*}

¹Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, Guangdong 510515, China

²Biosafety Level-3 Laboratory, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, Guangdong 510515, China

³State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong 510515, China

⁴Department of Human and Molecular Genetics, Virginia Commonwealth University, Richmond, VA 23298, USA

⁵Guangdong Provincial Key Laboratory of Proteomics, Southern Medical University, Guangzhou, Guangdong 510515, China

Abstract

Background & Aims—The macrophage scavenger receptor 1 (Msr1, also called SRA) is a pattern recognition receptor primarily expressed on myeloid cells, which plays an important role in the maintenance of immune homeostasis. Since MSR1 expression was upregulated in the livers of patients with fulminant hepatitis (FH), we investigated the functional mechanism of Msr1 in FH pathogenesis.

Methods—*Msr1*-deficient (*Msr1*^{−/−}) mice and their wild-type (WT) littermates were infected with mouse hepatitis virus strain-A59 (MHV-A59) to induce FH, and the levels of tissue damage, serum alanine aminotransferase, inflammatory cytokines and complement component 5a (C5a)

*Corresponding authors. Addresses: Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, Guangdong 510515, China. Tel.: +86 20 6164 8220; fax: +86 20 6164 8221 (Z. Chen), or Department of Immunology, School of Basic Medical Sciences, Southern Medical University, Guangzhou, Guangdong 510515, China. Tel.: +86 20 6164 8477 (D. Zuo). zhlchen@smu.edu.cn (Z. Chen), zdaming@smu.edu.cn (D. Zuo).

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors contributions

DZ, XYW, JH and ZC designed research. YT, HL, JL, Yanli Li, Yunzhi Liu, Jia Zhou, Jing Zhou, and XL performed experiments. YT, Jia Zhou, WZ, JH, ZC, XYW, and DZ analyzed data. DZ, ZC, and XYW wrote the manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jhep.2017.11.010>.

were measured and compared. Liver injury was studied after MHV infection with or without neutrophil depletion.

Results—Our results showed that *Msr1*^{−/−} mice were resistant to MHV-induced hepatitis. Treatment with the C5a receptor antagonist (C5aRa) diminished the differences in inflammatory responses and liver injury between MHV-infected wild-type and *Msr1*^{−/−} mice, suggesting that C5a-induced proinflammatory response plays a critical role in the Msr1-mediated regulation of FH pathogenesis. We demonstrated that Msr1 efficiently enhanced transforming growth factor-activated kinase-1 phosphorylation in neutrophils upon MHV-A59 stimulation, thereby promoting the activation of the extracellular signal-regulated kinase pathway and subsequent NETosis formation. Moreover, we provided evidence that blockage of Msr1 attenuated the liver damage caused by MHV-A59 infection.

Conclusions—Msr1 promotes the pathogenesis of virus-induced FH by enhancing induction of neutrophil NETosis and subsequent complement activation. Targeting Msr1 may be employed as a new immunotherapeutic strategy for FH.

Lay summary—Virus-induced fulminant hepatitis (FH) is a disease with a high mortality worldwide. Enhanced levels of macrophage scavenger receptor 1 (Msr1) in the liver of patients with FH and of murine experimental FH indicated Msr1 plays a role in the pathogenesis of FH. Herein, we demonstrate that mice deficient in Msr1 are resistant to FH induced by MHV-A59, and the Msr1 inhibitor fucoidan suppresses the progression of FH in mice. Our study suggests that use of drugs inhibiting MSR1 function could be beneficial to patients with FH.

Graphical Abstract

Keywords

Macrophage scavenger receptor 1; Complement activation; Hepatitis; Neutrophils; Neutrophil elastase; Fibrinogen-like protein 2

Introduction

Fulminant hepatitis (FH) is a rare but potentially fatal disease caused by viral infection or inflammatory destruction of liver tissue. It is characterized by rapid deterioration of hepatic functions, massive hepatocyte necrosis, and hepatic encephalopathy.¹ Despite the recent therapeutic advances, FH is still associated with significant mortality. Mouse hepatitis virus (MHV) is a positive-strand RNA virus that causes a variety of diseases, including hepatitis,

enteritis, and encephalitis in mice.² Noteworthy, the MHV-induced disease profiles are dependent on the viral strain and infection route, as well as genetic background and immune status of the mice.^{2,3} Intraperitoneal infection of susceptible strains of mice (*i.e.* BALB/cJ, C57BL/6) with hepatotropic MHV, such as MHV-3 and MHV-A59, results in sinusoidal thrombosis and hepatocellular necrosis, and thus, has served as a useful model for FH in humans.^{4,5}

Fibrin deposition and thrombosis within the microvasculature is now recognized to play a pivotal role in the hepatocellular injury in experimental and human FH.^{6,7} Indeed, fibrinogen-like protein 2 (Fgl2) has been shown previously to have serine protease activity capable of directly cleaving prothrombin to thrombin, leading to fibrin deposition.⁸ In the setting of mice with MHV infection, the induction of Fgl2 was seen in macrophages and endothelial cells in the liver, followed by fibrin deposition and liver necrosis.⁶ Strikingly, pretreatment with neutralizing antibodies against Fgl2 prevented the coagulation disturbance and hepatic necrosis, thereby reducing mortality in infected mice.⁹ In addition to Fgl2, accumulating evidence suggests that activation of immune cells and subsequent production of proinflammatory cytokines (*i.e.* tumour necrosis factor- α [TNF- α], interleukin [IL]-1 β , IL-6, and interferon- γ [IFN- γ]) play a critical role in the pathogenesis of FH.^{7,10–12} Moreover, MHV infection causes massive complement activation and elevation of pro-inflammatory complement component 5a (C5a) has been proposed to accelerate the development of FH.^{7,10} Therefore, understanding the immunological mechanisms of FH will provide opportunities to potentially develop more pervasive and efficient treatment of FH for the benefits of the patients.

The macrophage scavenger receptor 1 (Msr1), also known as scavenger receptor type A (SRA), is preferentially expressed on myeloid cells, such as macrophages and dendritic cells (DCs).¹³ Recently, Ozment *et al.* reported that Msr1 is present on circulating and bone marrow neutrophils and was upregulated in response to toll-like receptor 2 stimulation.¹⁴ Msr1 acts as an innate pattern recognition receptor capable of recognizing a broad spectrum of “self” and “non-self” ligands, it thereby plays an important role in host defense against foreign microbial infections and the maintenance of immune homeostasis.¹³ Importantly, emerging studies suggest that Msr1 participates in the pathogenesis of multiple inflammatory diseases and that its immunomodulatory function may be uncoupled from its endocytic/phagocytic activity.¹⁵ We previously found that interference of Msr1-mediated regulatory pathways resulted in hypersensitivity of mice to T cell-mediated hepatitis. Interestingly, transfer of *Msr1*-positive hepatic mononuclear cells or administration of Msr1 protein could decrease the severity of liver damage.¹⁶ However, little is known about the role of Msr1 in the pathogenesis of virus-induced FH.

In this study, we report a strong upregulation of Msr1 in human and murine experimental FH. Our results showed that *Msr1*-deficient mice were resistant to MHV-A59-induced FH, which was attributed to the suppressed inflammatory response and limited Fgl2 expression. Indeed, Msr1 enhanced induction of NETosis and release of neutrophil elastase (NE) from neutrophils upon MHV-A59 challenge. This promoted serum generation of C5a, which was associated with increased Fgl2 expression and consequent acute liver damage. Most importantly, Msr1 enhanced MHV-induced phosphorylation of the transforming growth

factor-activated kinase 1 (TAK1), thereby facilitating the extracellular signal-regulated kinase (ERK) signaling activation for induction of NETosis. Furthermore, we provided evidence that blockage of *Msr1* attenuated the liver damage caused by MHV-A59 infection, suggesting that inhibition of *Msr1*-mediated inflammatory response may be used as a new immunotherapeutic strategy for the treatment of FH.

Materials and methods

Patient samples

Paraffin-embedded liver tissues from fulminant liver failure ($n = 10$) were obtained from patients who were clinically, histologically and pathologically diagnosed for acute viral hepatitis with infection of hepatitis B virus in the Nanfang Hospital affiliated with Southern Medical University. The normal controls were adjacent liver tissues obtained from patients who underwent surgery for liver cancer ($n = 5$). All patients gave informed consent, and the experimental design and study were reviewed and approved by the Ethical Committee of Southern Medical University.

Mice and infection

Msr1-deficient (*Msr1*^{-/-}) mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). C57BL/6 mice were purchased from the Animal Institute of Southern Medical University (Guangzhou, China). The animals were maintained under a 12-h light/dark cycle in a specific pathogen-free animal facility at a controlled temperature (20–25 °C) and humidity (50 ± 5%). All mice were fed with regular diet and autoclaved water. All animal experiments in this study were approved by the Welfare and Ethical Committee for Experimental Animal Care of Southern Medical University.

Mice (male, 6–8 weeks old) were injected intraperitoneally with 7×10^5 plaque-forming unit (PFU) of MHV-A59 to generate survival curves in wild-type (WT) and *Msr1*^{-/-} mice. Administration of 5×10^5 PFU of MHV-A59 was used to assess liver damage and other parameters at different time points. To deplete liver macrophages, mice were intravenously injected with 200 µl of clodronate-containing liposomes (FormuMax Scientific, Palo Alto, CA, USA). Alternatively, neutrophil depletion was achieved by injecting 100 µg of anti-mouse lymphocyte antigen 6 complex locus G ([Ly6G] RB6-8C5, eBioscience, San Diego, CA, USA). In some experiments, WT or *Msr1*^{-/-} mice were injected intravenously with 1 mg/kg of C5a receptor antagonists (C5aRa) (GL Biochem, Shanghai, China) or injected intraperitoneally with 100 mg/kg of sivelestat (Sigma-Aldrich, St. Louis, MO, USA) 30 mins before MHV-A59 infection. To block the effect of *Msr1*, WT mice were injected intraperitoneally with 100 µg of fucoidan (Sigma-Aldrich) simultaneously with MHV-A59 administration.

Statistical analysis

The experimental data were evaluated by calculating the mean ± SD. One-way ANOVA was used for comparisons among multiple groups. Differences between two groups within experiments were analyzed by Student's *t* test. Comparison of the survival curves was performed using the Log-Rank test. *P* values <0.05 were considered statistically significant.

For further details regarding the materials used, please refer to the CTAT table and supplementary information.

Results

Hepatic expression of *Msr1* increases dramatically in human fulminant hepatitis and mouse model of fulminant hepatic failure

Our previous observation of an increased MSR1 expression in the liver from patients with autoimmune hepatitis and chronic type B hepatitis prompted us to assess hepatic MSR1 expression in patients with FH.¹⁶ Immunohistochemical staining showed that the level of MSR1 in the liver was highly elevated in patients with fulminant hepatic failure infected by hepatitis B virus (Fig. 1A). Similarly, hepatic *Msr1* expression increased significantly in the liver tissues from a mouse model of MHV-A59 induced fulminant hepatic failure, as indicated by immunohistochemical staining (Fig. 1B) and immunoblotting (Fig. 1C). We next examined the expression pattern of *Msr1* during FH. Fluorescence-activated cell sorting (FACS) analysis revealed that *Msr1* expression was increased on multiple subsets of mononuclear cells in the livers, including the CD11b⁺ leukocytes, CD11c⁺ DCs, F4/80⁺ macrophages and Ly6G⁺ neutrophils, upon MHV-A59 administration (Fig. 1D).

Mice deficient in *Msr1* are strongly resistant to MHV-A59 infection

To define the role of *Msr1* in virus-induced FH, WT and *Msr1*^{-/-} mice were injected intraperitoneally with 7×10⁵ PFU of MHV-A59, and the survival rate of mice was observed. Strikingly, all of the WT mice died within 48 h. However, 80% of *Msr1*^{-/-} mice survived the challenge (Fig. 2A). To further assess the effect of *Msr1* on liver injury, WT and *Msr1*^{-/-} mice were infected with 5×10⁵ PFU of MHV-A59, followed by serum and histology analysis at the indicated time points. The susceptibility of MHV-A59 is same for WT and *Msr1*^{-/-} mice upon intraperitoneal administration (Fig. S1A and S1B). Sera collected from MHV-A59- infected *Msr1*^{-/-} mice showed a lower level of alanine aminotransferase (ALT) activity than those from WT controls (Fig. 2B). MHV-A59-treated *Msr1*^{-/-} mice displayed limited hepatocyte necrosis and disseminated hemorrhage compared with those from WT counterparts (Fig. 2C). Besides, less terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive cells were found in the livers of MHV-A59- treated *Msr1*^{-/-} mice than those from WT mice (Fig. 2D).

Next, we compared the serum levels of inflammatory cytokines between MHV-A59 treated WT and *Msr1*^{-/-} mice to further determine the role of *Msr1* in MHV-A59-induced hepatitis. Attenuated liver damage in *Msr1*^{-/-} mice was accompanied by a pronounced reduction of pro-inflammatory cytokines (*i.e.* TNF-α, IL-6, and IFN-γ) and chemokine monocyte chemoattractant protein-1 (MCP-1), whereas increased secretion of the antiinflammatory cytokine IL-10 in serum (Fig. 2E). Accordingly, intrahepatic mRNA expression of the pro-inflammatory cytokines and chemokine was significantly suppressed in *Msr1*^{-/-} mice compared with those in WT mice upon MHV-A59 administration (Fig. 2F). Additionally, the change of *IL-10* mRNA was similar to that of protein level in serum between WT and *Msr1*^{-/-} mice (Fig. 2F). We then investigated the signaling pathways and found that the phosphorylation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B

P65 was attenuated in *Msr1*^{-/-} mice at different time points after MHV-A59 treatment (Fig. S2A and S2B). Collectively, these results indicated a potential role of Msr1 in promoting the pathogenesis of FH.

Msr1 controls coagulation and complement activation upon MHV-A59 infection

Hepatic Fgl2, a hallmark protein that causes necrosis of infected livers, is remarkably increased during the development of FH.⁶ To examine the effects of Msr1 on coagulation, we measured Fgl2 expression in the liver of mice. Enhanced *fgl2* transcript expression was determined by quantitative PCR, and its level in *Msr1*-deficient liver was lower than that in WT control following MHV-A59 infection (Fig. 3A). Immunohistochemical staining revealed that livers from virus-infected *Msr1*^{-/-} mice exhibited less Fgl2 expression than those from WT counterparts (Fig. 3B). Immunoblotting analysis verified the lower Fgl2 protein level in the livers of *Msr1*^{-/-} mice, compared to WT littermates following MHV-A59 infection (Fig. 3C). Consistently, MHV-infected *Msr1*^{-/-} mice displayed a remarkably decreased Fgl2 level in serum, assessed by ELISA analysis (Fig. 3D). These data indicate that *Msr1* potentiates Fgl2 production and activates coagulation during the pathogenesis of murine viral FH.

A recent study has shown that C5a/C5aR axis-mediated complement activation is responsible for the generation of Fgl2 during FH.¹⁰ Therefore, we measured the C5a production in mice upon MHV-A59 infection. As expected, MHV-A59 challenge led to a rapid increase in serum levels of C5a in both WT and *Msr1*^{-/-} mice. C5a production was significantly suppressed in *Msr1*^{-/-} mice compared with those in WT mice after MHV-A59 treatment (Fig. 3E). In agreement with this observation, Msr1 deficiency led to a significantly reduced C5a level in liver from MHV-A59-infected mice (Fig. 3F). Additionally, immunohistochemical staining and immunoblotting analysis showed that the level of hepatic C5aR was comparable between virus-infected WT and *Msr1*^{-/-} mice (Fig. S3A, B). Together, these results suggested that the complement cascade was less active in MHV-A59-infected *Msr1*^{-/-} mice than in WT mice.

Blockade of C5a/C5aR pathway eliminates the difference in liver damage between virus-infected WT and *Msr1*^{-/-} mice

To further investigate the role of complement activation in function of Msr1 during FH, WT and *Msr1*^{-/-} mice were pretreated with C5aRa or normal saline *via* intravenous injection before MHV-A59 administration. The severity of liver damage was comparable between MHV-A59-injected WT and *Msr1*^{-/-} mice upon C5aR blockade (Fig. 4A). The sera from WT and *Msr1*^{-/-} mice also showed no significant difference in the levels of ALT activity (Fig. 4B). Hepatic Fgl2 expression was similar between MHV-A59-challenged WT and *Msr1*^{-/-} mice upon interruption of C5a/C5aR pathway (Fig. 4C). Our data also showed that both WT and *Msr1*^{-/-} mice displayed substantially reduced production of pro-inflammatory cytokines in the absence of C5aR signals. Moreover, the levels of the cytokines were comparable between MHV-A59-infected WT and *Msr1*^{-/-} mice when C5a/C5aR pathway was blocked (Fig. 4D). These data suggest that Msr1 modulates Fgl2 expression and the development of murine FH through enhancing activation of the C5a/C5aR pathway and production of C5a.

Neutrophils from WT mice generate more C5a than those from *Msr1*^{-/-} mice after MHV-A59 challenge

The previous study demonstrated that phagocytic cells (*i.e.* macrophages and neutrophils) are able to cleave C5 into biologically active C5a.¹⁷ FACS analysis showed the infiltration of various subsets of mononuclear cells (*i.e.* CD11b⁺ leukocytes, CD11c⁺ DCs, F4/80⁺ macrophages and Ly6G⁺ neutrophils) into livers was comparable between WT and *Msr1*^{-/-} mice upon MHV-A59 administration (Fig. S4). Given that *Msr1* is expressed primarily on such cells,^{13,14} we raised the question of which cell-associated *Msr1* modulated the generation of C5a. Clodronate liposomes were employed to deplete the macrophage population *in vivo* (Fig. S5), and anti-Ly6G antibodies were chosen to deplete neutrophils in mice (Fig. S6). The MHV-A59-induced liver damage was lessened in *Msr1*^{-/-} mice in comparison with WT mice, treated with either clodronate liposomes or control liposomes, as indicated by reduced hepatic necrosis, ALT activity, C5a production and serum Fgl2 production (Fig. 5A, B). By contrast, depletion of neutrophils abolished the difference in liver damage between MHV-A59-infected WT and *Msr1*^{-/-} mice. No significant difference in liver pathology was observed between virus-treated WT and *Msr1*^{-/-} mice after neutrophil depletion (Fig. 5C). Also, serum ALT activity and the serum levels of C5a and Fgl2 were comparable between virus-treated WT and *Msr1*^{-/-} mice when neutrophils were depleted *in vivo* (Fig. 5D). Interestingly, the expression of *Msr1* in neutrophils was upregulated upon MHV-A59 stimulation *in vitro* determined by immunoblotting, suggesting that *Msr1* may affect the function of neutrophils (Fig. S7). Furthermore, we evaluated the complement activation induced by neutrophils in response to MHV infection. Neutrophils isolated from MHV-infected WT mice were significantly more potent than those from MHV-infected *Msr1*^{-/-} mice in cleaving C5 into C5a (Fig. 5E). Additionally, WT neutrophils had a stronger capability to cleave C5 than *Msr1*^{-/-} neutrophils upon MHV-A59 stimulation *in vitro* (Fig. 5F). Taken together, these findings indicated that *Msr1*-positive neutrophils were accountable for the pathogenesis of FH.

Augmented NETosis and increased neutrophil elastase are closely associated with *Msr1*-mediated regulation of FH progression

It has been reported that neutrophils have a distinct antimicrobial mechanism to trap and kill pathogens, which are so-called neutrophil extracellular traps (NETs).¹⁸ The release of NETs is the result of a specialized form of neutrophil death, dubbed NETosis.^{18,19} Here, we assessed the ability of MHV-A59 to induce NETosis in isolated murine neutrophils using the cell-impermeable DNA dye SYTOX green. Notably, more NETosis occurred in WT neutrophils compared to *Msr1*^{-/-} neutrophils upon MHV-A59 stimulation (Fig. 6A). We also examined the NETs formed by WT and *Msr1*^{-/-} neutrophils upon MHV-A59 stimulation using an immunofluorescence approach that showed colocalization of NETs components, myeloperoxidase (MPO) and NE. As shown in Fig. 6B, WT neutrophils produced many more NETs than *Msr1*^{-/-} neutrophils after virus treatment. During NETosis, the serine protease NE is present on NETs released from activated neutrophils, which plays a crucial role in host defense.²⁰ We observed an excessive amount of NE released from WT neutrophils compared to *Msr1*^{-/-} neutrophils after MHV-A59 stimulation *in vitro* (Fig. 6C). Strikingly, much higher activity of NE was shown in the serum from MHV-infected WT mice than that from MHV-infected *Msr1*^{-/-} mice (Fig. 6D). To further validate the function

of NE in the Msr1-mediated regulation of the pathogenesis of FH, WT and *Msr1*^{-/-} mice were treated with a NE inhibitor, sivelestat, before MHV-A59 infection, followed by histological and serum analysis. The severity of liver damage was comparable between WT and *Msr1*^{-/-} mice after sivelestat treatment (Fig. 6E). Additionally, the sera collected from WT and *Msr1*^{-/-} mice showed no significant difference in the levels of ALT activity and the productions of C5a and Fgl2 (Fig. 6F). These data suggest that Msr1 aggravated the pathogenesis of virus-induced FH by promoting neutrophils towards NETosis and release of NE.

TAK1 is involved in Msr1-enhanced NETosis formation upon MHV-A59 stimulation

Given the previously reported endocytic activity of Msr1,¹³ we first determined the potential impact of Msr1 on receptormediated endocytosis of MHV-A59. FACS analysis showed that WT and *Msr1*^{-/-} neutrophils exhibited similar fluorescence after incubation with fluorescein isothiocyanate (FITC)-labeled MHV-A59 (Fig. S8), indicating that Msr1 might serve as an immune modulator rather than endocytic/phagocytic receptor in the pathogenesis of FH. Recent evidence supports involvement of the Raf-ERK pathway in NETosis formation.²¹ To provide insights into the molecular mechanisms underlying Msr1-mediated immunoregulation, we investigated the activation of ERK pathways in WT and *Msr1*^{-/-} neutrophils after MHV stimulation. The increased phosphorylation of ERK was shown in MHV-A59-stimulated WT neutrophils compared to *Msr1*^{-/-} counterparts (Fig. 7A). Additionally, we also examined the type I interferon response in neutrophils during MHV stimulation. Both phosphorylation of interferon regulatory transcription factors, IRF3 and IRF7, as well as production of IFN- α was similar between WT and *Msr1*^{-/-} neutrophils after MHV-A59 treatment (Fig. S9). Furthermore, pretreatment with ERK inhibitor, U0126, substantially reduced formation of NETosis in both WT and *Msr1*^{-/-} neutrophils. Moreover, the formation of NETosis was comparable between MHV-A59-treated WT and *Msr1*^{-/-} neutrophils in these two groups when the ERK pathway was inhibited (Fig. 7B).

TAK1 is a known activator of MAPK signaling pathways in various immune cells, including neutrophils, and was also reported to act upstream of ERK.²² Therefore, we examined the possibility that TAK1 is involved in Msr1-mediated regulation of NETosis formation. Upon MHV-A59 stimulation, WT neutrophils displayed higher levels of TAK1 phosphorylation compared with *Msr1*^{-/-} cells (Fig. 7C). Next, WT and *Msr1*^{-/-} neutrophils were stimulated by MHV-A59 in the presence or absence of the TAK1 inhibitor, LL Z1640-2. First, we confirmed that LL Z1640-2 was able to inhibit TAK1 phosphorylation (Fig. 7D). Additionally, MHV-A59 induced ERK phosphorylation was suppressed by pretreatment of the TAK1 inhibitor under the same conditions, indicating that TAK1 activation is required for ERK activation after MHV stimulation (Fig. 7D). Notably, the formation of NETosis and activity of released NE were comparable in MHV-A59-infected WT and *Msr1*^{-/-} neutrophils upon TAK1 inhibition (Fig. 7E, F). Together, these data suggest that Msr1 enhances TAK1 phosphorylation upon MHV-A59 stimulation, which results in activation of the ERK pathway and consequent NETosis formation.

Blockade of Msr1 ameliorates MHV-A59-induced hepatic injury and complement activation

To directly examine the potential effect of Msr1 in FH, WT mice were intraperitoneally injected with a pharmacological inhibitor of Msr1, fucoidan,²³ simultaneously with MHV-A59 infection. *In vivo* injection of fucoidan was able to significantly prolong the survival of the virus-infected WT mice (Fig. 8A). Mice receiving fucoidan showed marked reduction of liver injury, as evidenced by reduced serum ALT levels (Fig. 8B) and hepatic pathology (Fig. 8C). Moreover, fucoidan inhibited the production of serum C5a upon MHV-A59 infection (Fig. 8D). Taking together, we conclude that the Msr1 plays a decisive role in regulating neutrophil-mediated complement activation, and thereby affects the severity of liver pathology and animal mortality during MHV infection.

Discussion

Msr1 exerts systemic effects in a variety of diseases, including atherosclerosis, sepsis, endotoxemia, and viral infections.²⁴ In this study, we used a mouse FH model induced by MHV-A59 infection to investigate the effects of Msr1 in this disease process. We demonstrate that Msr1 acts as an accelerator in MHV-induced inflammatory response. The alleviated disease observed in infected *Msr1*^{-/-} mice is associated with a substantial decrease in the production of Fgl2 and C5a, which are critical for the initiation of FH pathogenesis. Our results have revealed that the exacerbated effect of Msr1 on MHV-induced FH relies on the neutrophil activity. Moreover, we have provided the evidence that Msr1 promotes neutrophil NETosis triggered by MHV-A59 infection, enhancing the release of NE and the production of C5a, which further accelerates the pathogenesis of virus-induced FH.

Systemic inflammatory response syndrome has long been considered as an essential element in patients with fulminant hepatic failure.²⁵ The clinical syndrome is mediated by the release of pro- and anti-inflammatory cytokines into the systemic circulation from the liver. Subsequently, the accumulation of pro-inflammatory substances results in exacerbation of injury to the liver and other organs. The previous report showed that Msr1 has a regulatory function in cytokine production upon microbe infection. Msr1 potentiates the inflammatory response mediated by toll-like receptor 3 that is located in endomembranes by mediating pathogen internalization.²⁶ Alternatively, some studies suggested that macrophage-expressed Msr1 may limit pro-inflammatory cytokine production in response to mycobacteria, unrelated to its activity in pathogen phagocytosis.^{27,28} Therefore, regulation of Msr1 expression may be important for its functions under certain pathogenic conditions. Here, we demonstrate that Msr1 facilitates the pro-inflammatory cytokines in a mouse model of FH induced by MHV-A59 infection. Notably, Msr1-mediated increases in the production of pro-inflammatory cytokines upon MHV-A59 challenge is dependent on the induction of C5a/C5aR pathway because blockade of C5aR signaling abrogates the detrimental effect of Msr1 on viral FH. C5a is an active inflammatory component that initiates proinflammatory responses and activates numerous host immune effector cells, including macrophages, neutrophils and other immune cells.^{29,30} Our study shows dramatically increased serum C5a levels at the very early stage of virus-induced FH, in line with the previous report,¹⁰ indicating that C5a is an early mediator of the disease. Meanwhile, virus-infected *Msr1*^{-/-} mice display limited serum C5a levels compared to WT controls. Attenuation of FH in

Msr1^{-/-} mice correlates with decreased activation of MAPKs pathways and reduced induction of Fgl2, which are known major contributors to the pathogenesis of FH and can be induced by C5a stimulation.^{7,9,10,31} These data suggest that *Msr1*-facilitated production of C5a plays a crucial role in the development of FH.

Generally, conversion of C5 to C5a requires prior activation of complement component 3 (C3).³² However, the previous study showed that the attenuation of susceptibility to viral FH in C3-deficient mice was much milder compared with the C5aR-deficient mice, indicating that the generation of C5a is at least partially independent on C3 activation in the development of FH.¹⁰ Huber-Lang *et al.* showed that activated phagocytic cells (*i.e.* macrophages and neutrophils) can generate C5a from C5.¹⁷ In agreement with this observation, we have found that hepatic neutrophils can cleave C5 into C5a *in vitro* after MHV-A59 stimulation, which is also supported by a previous report that NE released from activated neutrophil has C5 convertase (C5agenerating) activity.³³ The intriguing function of *Msr1* in neutrophil-mediated C5a generation was also supported by our *in vivo* cell depletion study. Additionally, neutrophils from infected WT mice cleave more C5 to C5a compared with *Msr1*^{-/-} mice, whereas there was no difference in phagocytic activity between WT and *Msr1*^{-/-} neutrophils. Experimental evidence suggests that neutrophils might play a critical role in the pathogenesis of acute liver failure by contributing to the initiation and perpetuation of immune dysregulation through the formation of NETs, synthesis of pro-inflammatory cytokines and direct liver damage.^{34,35} In the context of viral infection, neutrophils undergo NETosis, a novel type of programmed cell death, and release NETs.³⁵ Our current work demonstrates that *Msr1* promotes the NETosis in neutrophils upon MHV stimulation, whereas Liao *et al.* reported that expression of *Msr1* conferred resistance of macrophages to the apoptotic stimulation by oxLDL and its cytotoxic lipid component.³⁶ These two seemingly distinct aspects of *Msr1* activity are probably indications of its functional flexibility in mediating differential cellular events. *Msr1* expressed on differential cells may adapt to specific cellular needs in response to different types of stimulus.

NETs are described as an antimicrobial mechanism, based on the fact that NETs allow neutrophils to deliver high concentrations of cytotoxic agents (*i.e.* reactive oxygen species, antimicrobial peptides, and proteases such as cathepsin G, NE and proteinase 3) that degrade virulence factors and kill bacteria extracellularly.^{18,35} Recent findings indicate the importance of neutrophil serine proteases as key mediators of inflammatory processes in neutrophil-mediated tissue injury.^{18,20} Massberg *et al.* demonstrated that neutrophil serine proteases in concert with externalized nucleosomes initiate coagulation as a host defense mechanism.³⁷ Neutrophil initiated coagulation is probably the result of a complement-dependent mechanism, as shown by Pagano *et al.*³⁸ for the development of the elastase-induced abdominal aortic aneurysm. Moreover, neutrophil serine proteases operate as important activators of TNF- α , IL-1 β , and IL-18 to enhance the immune response.³⁹ Takai *et al.*⁴⁰ reported that NE contributes to the early step of the inflammatory cascade in acute viral hepatitis and NE inhibitor reduces the expression of inflammatory cytokines and chemokines in the liver, attenuating liver injury in hepatitis B transgenic mice. In agreement with these reports, our finding of *Msr1*-increased hypersensitivity to FH correlates with excessive NETosis and subsequently a high release of NE. Therefore, the discrepancy in the

severity of FH in WT and *Msr1*^{-/-} mice may be attributed to the different levels of NETosis induced in neutrophils upon MHV-A59 administration.

A recent study showed that the Raf-ERK pathway is involved in NETosis induction through activation of NADPH oxidase and upregulation of anti-apoptotic proteins.²¹ TAK1 can be linked to ERK pathway in human neutrophils.⁴⁰ Here, we report for the first time, as far as we know, that TAK1 is involved in ERK activation and subsequent NETosis induction in the pathogenesis of FH. Furthermore, *Msr1* contributes to TAK1 activation, which therefore delivers a signal for NET formation. A recent study revealed that cross-linking of FcγRIIIb activates TAK1 and triggers the ERK signaling pathway, leading to induction of NETosis.²² This study also indicates that spleen tyrosine kinase (Syk) is required for TAK1 activation and subsequent NET formation after FcγRIIIb engagement.²² Notably, the activation of Syk family kinases occurs after the crosslink of activating FcγRs with Src family kinases, such as Fyn, Lck, or Lyn.⁴¹ Miki *et al.* demonstrated that *Msr1* directly interacts with Lyn kinase,⁴² and the engagement of *Msr1* also caused the activation of both Lyn and phosphoinositide 3-kinase.⁴³ It is possible that *Msr1* modulates MHV infection-induced TAK1 phosphorylation by regulating the sequential activation of Lyn and Syk kinases. Indeed, FcγRIIIb expressed in monocytes/macrophages exhibits scavenger receptor-like function and has been implicated in inflammatory processes associated with atherosclerosis.⁴⁴ Taken together, we postulate that the co-expression of *Msr1* with FcγRIIIb on inflammatory cells may be functionally complementary to each other, *via* Src family kinases, modulating the pro-inflammatory responses and subsequent progression of diseases.

In summary, our data elucidate an important feature of *Msr1* in the exacerbation of mouse viral FH, suggesting that interference of MSR1-mediated regulatory pathways may be a potential therapeutic strategy for patients with FH. Moreover, our findings substantiate a concept that increased *Msr1* expression on neutrophils leads to over-activation of the complement system during infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support

This work was supported in part by Foundation for Distinguished Young Teacher in Higher Education of Guangdong (grant no.: Yq2013034), National Natural Science Foundation of China (grant nos.: 31370875 and 81671568), Science and Technology Planning Project of Guangdong Province (grant no.: 2016A020215106), Science and Technology Planning Project of Guangzhou (grant no.: 201607010195), and National Institutes of Health Grants CA175033.

References

Author names in bold designate shared co-first authorship

1. Polson J, Lee WM. American Association for the Study of Liver Disease. AASLD position paper: the management of acute liver failure. *Hepatology*. 2005; 41:1179–1197. [PubMed: 15841455]

2. Eriksson KK, Cervantes-Barragan L, Ludewig B, Thiel V. Mouse hepatitis virus liver pathology is dependent on ADP-ribose-1''-phosphatase, a viral function conserved in the alpha-like supergroup. *J Virol.* 2008; 82:12325–12334. [PubMed: 18922871]
3. Homberger FR. Enterotropic mouse hepatitis virus. *Lab Anim.* 1997; 31:97–115. [PubMed: 9175007]
4. Aftring RP, Freeman MW. Structure of the murine macrophage scavenger receptor gene and evaluation of sequences that regulate expression in the macrophage cell line, P388D. *J Lipid Res.* 1995; 36:1305–1314. [PubMed: 7666008]
5. Levy GA, Leibowitz JL, Edgington TS. Induction of monocyte procoagulant activity by murine hepatitis virus type 3 parallels disease susceptibility in mice. *J Exp Med.* 1981; 154:1150–1163. [PubMed: 6270227]
6. Marsden PA, Ning Q, Fung LS, Luo X, Chen Y, Mendicino M, et al. The Fgl2/fibroleukin prothrombinase contributes to immunologically mediated thrombosis in experimental and human viral hepatitis. *J Clin Invest.* 2003; 112:58–66. [PubMed: 12840059]
7. Liu J, Tan Y, Zhang J, Zou L, Deng G, Xu X, et al. C5aR, TNF-alpha, and FGL2 contribute to coagulation and complement activation in virus-induced fulminant hepatitis. *J Hepatol.* 2015; 62:354–362. [PubMed: 25200905]
8. Levy GA, Liu M, Ding J, Yuwaraj S, Leibowitz J, Marsden PA, et al. Molecular and functional analysis of the human prothrombinase gene (HFGL2) and its role in viral hepatitis. *Am J Pathol.* 2000; 156:1217–1225. [PubMed: 10751347]
9. Li C, Fung LS, Chung S, Crow A, Myers-Mason N, Phillips MJ, et al. Monoclonal antiprothrombinase (3D4. 3) prevents mortality from murine hepatitis virus (MHV-3) infection. *J Exp Med.* 1992; 176:689–697. [PubMed: 1324969]
10. Xu GL, Chen J, Yang F, Li GQ, Zheng LX, Wu YZ. C5a/C5aR pathway is essential for the pathogenesis of murine viral fulminant hepatitis by way of potentiating Fgl2/fibroleukin expression. *Hepatology.* 2014; 60:114–124. [PubMed: 24604562]
11. Sekiyama KD, Yoshida M, Thomson AW. Circulating proinflammatory cytokines (IL-1 beta, TNF-alpha, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol.* 1994; 98:71–77. [PubMed: 7923888]
12. Wang JY, Wang XL, Liu P. Detection of serum TNF-alpha, IFN-beta, IL-6 and IL-8 in patients with hepatitis B. *World J Gastroenterol.* 1999; 5:38–40. [PubMed: 11819382]
13. Peiser L, Mukhopadhyay S, Gordon S. Scavenger receptors in innate immunity. *Curr Opin Immunol.* 2002; 14:123–128. [PubMed: 11790542]
14. Ozment TR, Graves B, Kalbfleisch J, Williams DL. Neutrophil scavenger receptor class A (SRA) expression is increased by TLR2 stimulation. *Shock.* 2014; 41:P52.
15. Amiel E, Acker JL, Collins RM, Berwin B. Uncoupling scavenger receptor A-mediated phagocytosis of bacteria from endotoxic shock resistance. *Infect Immun.* 2009; 77:4567–4573. [PubMed: 19667044]
16. Zuo D, Yu X, Guo C, Wang H, Qian J, Yi H, et al. Scavenger receptor A restrains T-cell activation and protects against concanavalin A-induced hepatic injury. *Hepatology.* 2013; 57:228–238. [PubMed: 22821642]
17. Huber-Lang M, Younkin EM, Sarma JV, Riedemann N, McGuire SR, Lu KT, et al. Generation of C5a by phagocytic cells. *Am J Pathol.* 2002; 161:1849–1859. [PubMed: 12414531]
18. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science.* 2004; 303:1532–1535. [PubMed: 15001782]
19. Kaplan MJ, Radic M. Neutrophil extracellular traps: double-edged swords of innate immunity. *J Immunol.* 2012; 189:2689–2695. [PubMed: 22956760]
20. Meyer-Hoffert U, Wiedow O. Neutrophil serine proteases: mediators of innate immune responses. *Curr Opin Hematol.* 2011; 18:19–24. [PubMed: 21042214]
21. Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nat Chem Biol.* 2011; 7:75–77. [PubMed: 21170021]

22. Aleman OR, Mora N, Cortes-Vieyra R, Uribe-Querol E, Rosales C. Transforming growth factor-beta-activated kinase 1 is required for human FcγRIIIb-induced neutrophil extracellular trap formation. *Front Immunol.* 2016; 7:277. [PubMed: 27486461]
23. Yokota T, Ehlin-Henriksson B, Hansson GK. Scavenger receptors mediate adhesion of activated B lymphocytes. *Exp Cell Res.* 1998; 239:16–22. [PubMed: 9511720]
24. Kelley JL, Ozment TR, Li C, Schweitzer JB, Williams DL. Scavenger receptor-A (CD204): a two-edged sword in health and disease. *Crit Rev Immunol.* 2014; 34:241–261. [PubMed: 24941076]
25. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. *Hepatology.* 2000; 32:734–739. [PubMed: 11003617]
26. Dansako H, Yamane D, Welsch C, McGivern DR, Hu F, Kato N, et al. Class A scavenger receptor 1 (MSR1) restricts hepatitis C virus replication by mediating toll-like receptor 3 recognition of viral RNAs produced in neighboring cells. *PLoS Pathog.* 2013; 9:e1003345. [PubMed: 23717201]
27. Hollifield M, Bou Ghanem E, de Villiers WJ, Garvy BA. Scavenger receptor A dampens induction of inflammation in response to the fungal pathogen *Pneumocystis carinii*. *Infect Immun.* 2007; 75:3999–4005. [PubMed: 17548480]
28. Baer MT, Huang N, Gibson FC 3rd. Scavenger receptor A is expressed by macrophages in response to *Porphyromonas gingivalis*, and participates in TNF-α expression. *Oral Microbiol Immunol.* 2009; 24:456–463. [PubMed: 19832797]
29. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol.* 2009; 9:729–740. [PubMed: 19730437]
30. Cheng SC, Sprong T, Joosten LA, van der Meer JW, Kullberg BJ, Hube B, et al. Complement plays a central role in *Candida albicans*-induced cytokine production by human PBMCs. *Eur J Immunol.* 2012; 42:993–1004. [PubMed: 22531923]
31. Chiou WF, Tsai HR, Yang LM, Tsai WJ. C5a differentially stimulates the ERK1/2 and p38 MAPK phosphorylation through independent signaling pathways to induced chemotactic migration in RAW264. 7 macrophages. *Int Immunopharmacol.* 2004; 4:1329–1341. [PubMed: 15313431]
32. Noris M, Remuzzi G. Overview of complement activation and regulation. *Semin Nephrol.* 2013; 33:479–492. [PubMed: 24161035]
33. Ward PA, Hill JH. C5 chemotactic fragments produced by an enzyme in lysosomal granules of neutrophils. *J Immunol.* 1970; 104:535–543. [PubMed: 4985169]
34. Taylor NJ, Nishtala A, Manakkat Vijay GK, Abeles RD, Auzinger G, Bernal W, et al. Circulating neutrophil dysfunction in acute liver failure. *Hepatology.* 2013; 57:1142–1152. [PubMed: 23079896]
35. Yipp BG, Kubes P. NETosis: how vital is it? *Blood.* 2013; 122:2784–2794. [PubMed: 24009232]
36. Liao HS, Kodama T, Geng YJ. Expression of class A scavenger receptor inhibits apoptosis of macrophages triggered by oxidized low density lipoprotein and oxysterol. *Arterioscler Thromb Vasc Biol.* 2000; 20:1968–1975. [PubMed: 10938019]
37. Massberg S, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med.* 2010; 16:887–896. [PubMed: 20676107]
38. Pagano MB, Zhou HF, Ennis TL, Wu X, Lambris JD, Atkinson JP, et al. Complement-dependent neutrophil recruitment is critical for the development of elastase-induced abdominal aortic aneurysm. *Circulation.* 2009; 119:1805–1813. [PubMed: 19307471]
39. Pham CT. Neutrophil serine proteases fine-tune the inflammatory response. *Int J Biochem Cell Biol.* 2008; 40:1317–1333. [PubMed: 18180196]
40. Takai S, Kimura K, Nagaki M, Satake S, Kakimi K, Moriwaki H. Blockade of neutrophil elastase attenuates severe liver injury in hepatitis B transgenic mice. *J Virol.* 2005; 79:15142–15150. [PubMed: 16306586]
41. Rosales C. Fcγ receptor heterogeneity in leukocyte functional responses. *Front Immunol.* 2017; 8:280. [PubMed: 28373871]
42. Miki S, Tsukada S, Nakamura Y, Aimoto S, Hojo H, Sato B, et al. Functional and possible physical association of scavenger receptor with cytoplasmic tyrosine kinase Lyn in monocytic THP-1-derived macrophages. *FEBS Lett.* 1996; 399:241–244. [PubMed: 8985154]

43. Nikolic DM, Cholewa J, Gass C, Gong MC, Post SR. Class A scavenger receptor-mediated cell adhesion requires the sequential activation of Lyn and PI3-kinase. *Am J Physiol Cell Physiol*. 2007; 292:C1450–C1458. [PubMed: 17192284]
44. Zhu X, Ng HP, Lai YC, Craig JK, Nagilla PS, Raghani P, et al. Scavenger receptor function of mouse Fcγ receptor III contributes to progression of atherosclerosis in apolipoprotein E hyperlipidemic mice. *J Immunol*. 2014; 193:2483–2495. [PubMed: 25038257]

Highlights

- Msr1 expression is elevated in patients with FH and in a mouse model of FH induced by MHV-A59 infection.
- Mice deficient in *Msr1* are resistant to MHV-A59-induced FH.
- Msr1 aggravates the pathogenesis of virus-induced FH by promoting neutrophils towards NETosis and further complement activation.
- Msr1 efficiently enhances activation of TAK1 and ERK in neutrophils upon MHV-A59 stimulation, which results in NETosis formation.
- Msr1 inhibitor fucoidan can protect mice from MHV-induced FH.

Fig. 1. Upregulation of Msr1 in human fulminant hepatitis and the mice model of fulminant hepatic failure

(A) MSR1 expression in human liver tissues was detected by immunohistochemical staining.

(B–D) WT mice ($n = 5$) were infected with 5×10^5 PFU of MHV-A59. Hepatic Msr1 expression at different time points was evaluated using immunohistochemical staining (B) and immunoblotting analysis (C). The expression pattern of Msr1 on liver mononuclear cells, including the CD11b⁺ leukocytes, CD11c⁺ DCs, F4/80⁺ macrophages and Ly6G⁺ neutrophils, from those infected WT mice were measured by FACS analysis (D). Scale bar = 50 μ m. * $p < 0.05$, ** $p < 0.01$, determined by one-way ANOVA and unpaired Student's t test. The data shown represent three independent experiments. DC, dendritic cells; FACS, fluorescence-activated cell sorting; FH, fulminant hepatitis; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HC, HCC patient control; Ly6G, lymphocyte antigen 6 complex locus G; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; PFU, plaque-forming unit; WT, wild-type.

Fig. 2. *Msr1* ablation renders mice more resistant to MHV-A59 challenge

(A) WT and *Msr1*^{-/-} mice (*n* = 7) were injected with 7×10^5 PFU of MHV-A59 and overall survival of mice was monitored. Comparison of the survival rate was performed using the Log-Rank test. (B–F) 5×10^5 PFU of MHV-A59 was injected into WT and *Msr1*^{-/-} mice (*n* = 5). (B) Serum ALT activities were assessed at different time points after MHV-A59 infection. (C) 24 h after MHV-A59 injection, histological analysis of mouse livers was performed using H&E staining. Bottom panels show the higher-magnification views of the tissue damage area. Scale bars (top) = 100 μm, (bottom) = 50 μm. (D) The apoptosis in mice liver tissues was evaluated by TUNEL staining. Scale bars = 50 μm. (E) Serum levels of TNF-α, IL-6, IFN-γ, MCP-1, and IL-10 were determined at indicated time point post MHV

administration. (F) Relative intrahepatic mRNA expression levels of *TNF- α* , *IL-6*, *IFN- γ* , *MCP-1*, and *IL-10* were measured by quantitative RT-PCR and expressed as a ratio to *GAPDH*. * $p < 0.05$, ** $p < 0.01$, determined by unpaired Student's t test. One of the three independent experiments is shown. ALT, alanine aminotransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; PFU, plaque-forming unit; RT-PCR, reverse transcription PCR; TNF, tumour necrosis factor; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; WT, wild-type.

Fig. 3. *Msr1* enhances the activation of coagulation and complement in response to MHV-A59 infection

WT and *Msr1*^{-/-} mice (n = 5) were infected with 5×10^5 PFU of MHV-A59. (A) mRNA level of *fgl2* in the liver tissues was detected by quantitative RT-PCR analysis and expressed as a ratio to GAPDH. (B, C) Protein level of Fgl2 in the liver tissues was detected by (B) immunohistochemical staining and (C) western blotting. (D) Serum Fgl2 levels were measured by ELISA. (E, F) C5a levels were measured by ELISA in (E) serum and (F) liver grinding fluid from virus-infected WT and *Msr1*^{-/-} mice. Scale bars = 50 μ m. * p < 0.05, ** p < 0.01, determined by unpaired Student's *t* test. Data are representative of three independent experiments with similar results. Fgl2, fibrinogen-like protein 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; PFU, plaque-forming unit; RT-PCR, reverse transcription PCR; WT, wild-type.

Fig. 4. Blocking C5a/C5aR pathway eliminates the damage difference between virus-infected WT and *Msr1*^{-/-} mice

WT and *Msr1*^{-/-} mice (n = 5) were injected intravenously with 1 mg/kg C5aRa 30 min before MHV-A59 infection and treatment with saline as a mock control. (A) C5aRa-treated WT and *Msr1*^{-/-} mice displayed reduced and comparable liver damage, determined by H&E staining. (B) Serum ALT activities were measured at 48 h post MHV-A59 infection. (C) Fgl2 expression in the liver tissues was detected by western blotting. (D) Serum levels of TNF-α, IL-6, and IFN-γ were evaluated by ELISA at indicated time points following MHV-A59 administration. Scale bars = 100 μm. **p* < 0.05, ***p* < 0.01, N.S., not significant, determined by unpaired Student's *t* test. The data represent three independent experiments with similar results. ALT, alanine aminotransferase; C5a, complement component 5a; C5aR; C5a receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; IL, interleukin; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; TNF, tumour necrosis factor; WT, wild-type.

Fig. 5. Neutrophils but not macrophages are responsible for the difference in liver damage between WT and *Msr1*^{-/-} mice upon MHV-A59 challenge

(A, B) WT and *Msr1*^{-/-} mice (n = 5) were intravenous injected with 200 μ l clodronate liposomes or control liposomes without clodronate 1 day before MHV-A59 injection. (A) Mice were examined at 24 h after virus infection for liver histology. (B) Serum ALT activity as well as the serum levels of C5a and Fgl2 was measured at 24 h post MHV administration. (C, D) WT and *Msr1*^{-/-} mice (n = 5) were intraperitoneal injected with 100 μ g anti-mouse Ly6G antibody or rat IgG2b isotype control at 6 h following MHV-A59 infection. Anti-mouse Ly6G antibody-treated WT and *Msr1*^{-/-} mice displayed parallel liver damage upon MHV-A59 infection. (C) Mice were examined at 24 h after virus infection for liver histology. (D) Serum ALT activity, as well as the serum levels of C5a and Fgl2, were measured at 24 h post MHV administration. (E) Neutrophils isolated from the liver of virus-infected WT and *Msr1*^{-/-} mice respectively, then co-cultured with C5 for indicated time. C5a production in the culture supernatants was measured by ELISA. (F) Neutrophils isolated from uninfected WT and *Msr1*^{-/-} mice were co-cultured with MHV-A59 in the presence of C5. C5a production in the culture supernatant was measured by ELISA at the indicated time points. Scale bars = 100 μ m. **p* < 0.05, ***p* < 0.01, N.S., not significant, determined by unpaired Student's *t* test. The data represent three independent experiments with similar results. ALT, alanine aminotransferase; C5a, complement component 5a; Fgl2, fibrinogen-like protein 2; Ly6G, lymphocyte antigen 6 complex locus G; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; WT, wild-type.

Fig. 6. *Msr1* promotes NETosis and release of NE by neutrophils upon MHV-A59 challenge (A–C) Neutrophils isolated from WT or *Msr1*^{-/-} mice were stimulated with MHV-A59 *in vitro*. (A) The induction of NETosis was detected at 3 h after virus stimulation by fluorescent microscopy using SYTOX Green, Scale bars = 200 μ m. (B) The formation of NETs at 3 h after virus stimulation was visualized by staining of MPO (green) and NE (red), respectively. (C) NE activity in the cultured supernatant was evaluated at different time points. (D) NE activity in serum of infected WT and *Msr1*^{-/-} mice was measured at 12, 24 and 48 h post MHV-A59 injection. (E–F) WT and *Msr1*^{-/-} mice were injected intraperitoneally with the sivelestat or normal saline at 30 min before MHV-A59 injection. (E) Histological analysis of mice livers was performed using H&E staining at 24 h after virus infection. (F) The activity of serum ALT, as well as production of C5a and Fg12, was measured at 12, 24 and 48 h post MHV-A59 injection. Scale bars = 100 μ m. * p < 0.05, ** p < 0.01, N.S., not significant, determined by unpaired Student's *t* test. The data represent three independent experiments with similar results. C5a, complement component 5a; MHV, mouse hepatitis virus; MPO, myeloperoxidase; MSR1, macrophage scavenger receptor 1; NE, neutrophil elastase; NE, neutrophil extracellular traps; WT, wild-type.

Fig. 7. *Msr1* enhances NETosis via modulating the TAK1-ERK axis in neutrophils upon MHV-A59 stimulation

(A) Levels of phospho-ERK and total ERK in neutrophils from WT and *Msr1*^{-/-} mice were examined by immunoblotting at indicated time points after MHV-A59 infection. (B) Neutrophils isolated from WT and *Msr1*^{-/-} mice were pre-incubated with the ERK inhibitor U1026 followed by MHV-A59 treatment. The induction of NETosis was detected at 3 h after virus stimulation by fluorescent microscopy using SYTOX Green. Scale bar = 200 μ m. (C) Levels of phospho-TAK1 in neutrophils from WT and *Msr1*^{-/-} mice were examined by immunoblotting at indicated time points after MHV-A59 infection. (D–F) Neutrophils isolated from WT and *Msr1*^{-/-} mice were pre-incubated with the TAK1 inhibitor LLZ1640-2 followed by MHV-A59 stimulation. (D) Levels of phospho-ERK and phospho-TAK1 in neutrophils from WT and *Msr1*^{-/-} mice were examined by immunoblotting at indicated time points after MHV-A59 infection. (E) The induction of NETosis was detected at 3 h after virus stimulation by fluorescent microscopy using SYTOX Green. Scale bar = 200 μ m. (F) NE activity in the cultured supernatant was evaluated at the indicated time points after MHV-A59 infection. N.S., not significant, determined by unpaired Student's *t* test. The data represent three independent experiments with similar results. ERK1, extracellular signal-regulated kinase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MHV, mouse hepatitis virus; MPO, myeloperoxidase; MSR1, macrophage scavenger receptor 1; NE, neutrophil elastase; NE, neutrophil extracellular traps; TAK1, transforming growth factor-activated kinase-1; WT, wild-type.

Fig. 8. Blockage of Msr1 ameliorated MHV-A59-induced FH and inhibited complement activation

(A) WT mice ($n = 7$) were intraperitoneally injected with Msr1 inhibitor fucoidan (100 $\mu\text{g}/\text{per mouse}$) or normal saline simultaneously with 7×10^5 PFU of MHV-A59 virus. Overall survival of mice was monitored. Comparison of the survival rate was performed using the Log-Rank test. (B–D) WT mice ($n = 5$) were intraperitoneally injected with fucoidan or normal saline simultaneously with 5×10^5 PFU of MHV-A59. (B) ALT levels, (C) liver histology and (D) serum C5a were examined at 24 h after MHV-A59 injection. Scale bar = 100 μm . * $p < 0.05$, ** $p < 0.01$, determined by unpaired Student's t test. The data represent three independent experiments with similar results. ALT, alanine aminotransferase; C5a, complement component 5a; FH, fulminant hepatitis; MHV, mouse hepatitis virus; MSR1, macrophage scavenger receptor 1; PFU, plaque-forming unit; WT, wild-type.