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MERS-CoV infection causes brain damage in human DPP4-transgenic mice through complement-mediated inflammation

Yuting Jiang[1]†, Yuehong Chen[1]†, Hong Sun[2]†, Xiaolu Zhang[1], Lei He[1], Jiangfan Li[1], Guangyu Zhao[1,*] and Shihui Sun[1,*]

Abstract

The highly pathogenic Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a severe respiratory virus. Recent reports indicate additional central nervous system (CNS) involvement. In this study, human DPP4 transgenic mice were infected with MERS-CoV, and viral antigens were first detected in the midbrain-hindbrain 4 days post-infection, suggesting the virus may enter the brainstem via peripheral nerves. Neurons and astrocytes throughout the brain were infected, followed by damage of the blood brain barrier (BBB), as well as microglial activation and inflammatory cell infiltration, which may be caused by complement activation based on the observation of deposition of complement activation product C3 and high expression of C3a receptor (C3aR) and C5a receptor (C5aR1) in neurons and glial cells. It may be concluded that these effects were mediated by complement activation in the brain, because of their reduction resulted from the treatment with mouse C5aR1-specific mAb. Such mAb significantly reduced nucleoprotein expression, suppressed microglial activation and decreased activation of caspase-3 in neurons and p38 phosphorylation in the brain. Collectively, these results suggest that MERS-CoV infection of CNS triggers complement activation, leading to inflammation-mediated damage of brain tissue, and regulating of complement activation could be a promising intervention and adjunctive treatment for CNS injury by MERS-CoV and other coronaviruses.

INTRODUCTION

The highly pathogenic Middle East respiratory syndrome coronavirus (MERS-CoV) was first documented in the Middle East in 2012 [1]. As of 31 January 2020, 2519 cases had been reported by the World Health Organization, including 866 fatalities (https://www.who.int/csr/don/24-february-2020-mers-saudi-arabia/en/). The mortality rate of MERS-CoV is as high as 34%, far higher than that of the severe acute respiratory syndrome coronavirus (SARS-CoV) (8–10%) [2] or SARS-CoV-2 (1–8.6% in the 20 most affected countries, [https://coronavirus.jhu.edu/data/mortality]). MERS-CoV, like SARS-CoV, is a positive-strand RNA virus belonging to the C lineage within the *Betacoronavirus* genus [3]. Clinically, patients with MERS-CoV infections present with symptoms typical of coronavirus infections, such as severe respiratory infection, often with considerable extrapulmonary organ dysfunction. However, clinical and experimental studies have demonstrated that coronavirus infections also involve the CNS [4, 5].

A brain necropsy study confirmed that SARS-CoV infects neurons in the cortex and hypothalamus, resulting in oedema and scattered red-stained cells [6]. SARS-CoV infections also cause neuronal death in transgenic mice expressing human angiotensin-converting enzyme 2 [7]. Recently, data from Chan *et al.* [8] indicated that MERS-CoV infects human neuronal cells, and symptoms of neurologic injury have also been reported for MERS patients [9–12]. Thus, MERS-CoV may produce neurologic complications similar to those observed with SARS-CoV. However, such neurologic involvement may be masked in patients with MERS-CoV infection who are sedated for the management of severe acute respiratory distress syndrome

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Author affiliations: [1]State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, PR China; [2]Department of Basic Medical Sciences, North China University of Science and Technology, Tangshan 063210, PR China.
***Correspondence:** Shihui Sun, sunsh01@163.com; Guangyu Zhao, guangyu0525@163.com
Keywords: complement system; MERS-CoV; neurologic injury; transgenic mice.
Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; BBB, blood brain barrier; CNS, central nervous system; COVID-19, coronavirus disease 2019; CPE, cytopathic effects; DPP4, dipeptidyl peptidase 4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; H&E, hematoxylin and eosin; IBA-1, ionized calcium binding adaptor molecule 1; mAb, monoclonal antibody; MAC, membrane attack complex; MAPK, mitogen-activated protein kinase; MERS-CoV, Middle East respiratory syndrome coronavirus; NK, natural killer; NP, nuclear protein; pAb, polyclonal antibody; PBS, phosphate-buffered saline; SARS-CoV, severe acute respiratory syndrome coronavirus; TCID50, 50% tissue culture infectious dose.
†These authors contributed equally to this work
Two supplementary figures are available with the online version of this article.

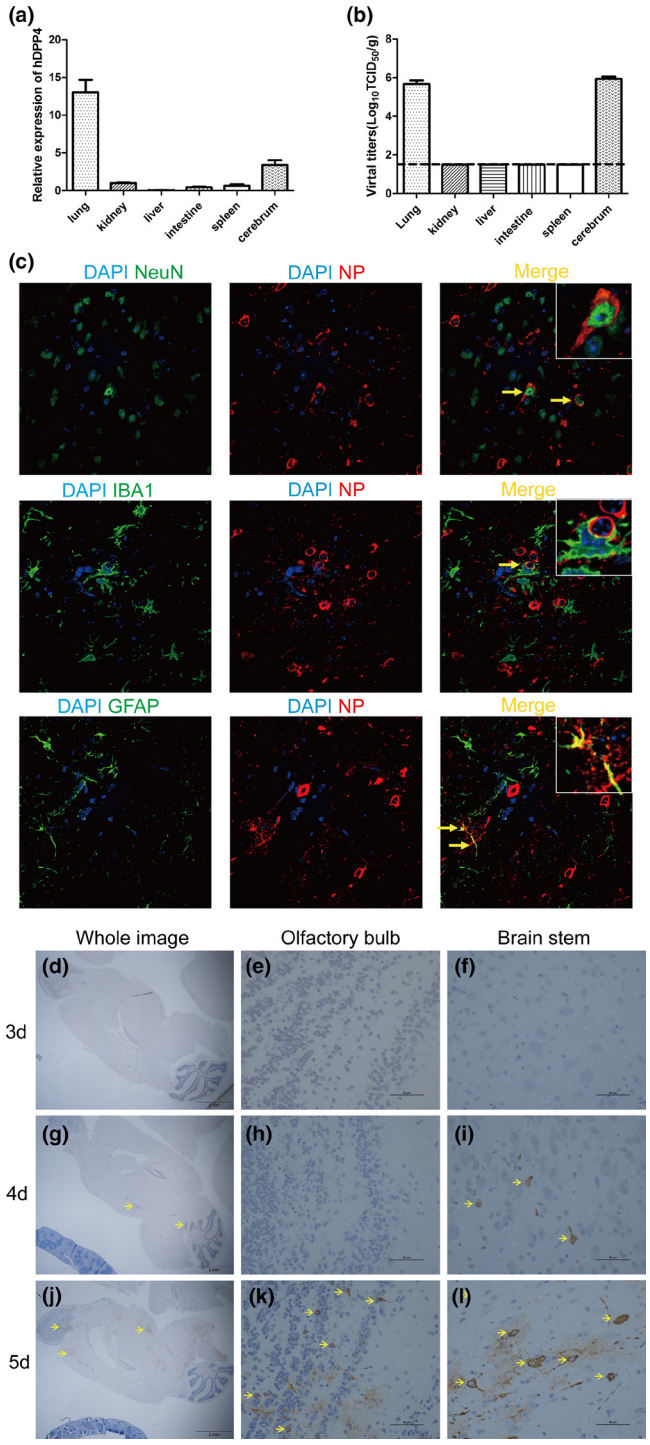

Fig. 1. MERS-CoV infection of brain tissues in hDPP4-transgenic mice. (a) Relative expression of hDPP4 in different tissues of hDPP4 transgenic mice. (b) Viral titres in different tissues of hDPP4 transgenic mice 7 days after infection with MERS-CoV (n=4–5 per group). (c) Double immunofluorescence staining of viral antigen (NP) and NeuN, IBA-1, or GFAP in brain sections from hDPP4 transgenic mice 7 days after infection with MERS-CoV (n=3 per group, magnification: 600×). (d–l) Representative images of immunohistochemical staining of MERS-CoV antigen in brains of hDPP4 transgenic mice 3, 4, and 5 days after virus infection. The viral antigen was first detected (yellow arrows) in the brainstem on day 4 post-infection (g–i) and later detected in the olfactory bulbs and cerebral cortex on day 5 (j–l) (n=3 per group).

and may remain undetected given the lack of brain necropsies for fatal cases.

A functional receptor of MERS-CoV is dipeptidyl peptidase 4 (DPP4), which is widely expressed on T cells and in the lung, kidney, liver, heart, placenta, and brain [13]. The widespread expression and multifunctional roles of DPP4 in apoptosis and immune responses may explain the impact of MERS-CoV both in the brain and on extrapulmonary organ dysfunction. The amino acid sequence for DPP4 is conserved among species and its specificity is likely a major factor in the species tropism of MERS-CoV [14, 15]. Common marmosets have been considered a suitable nonhuman primate model based on the effective interaction of its DPP4 with the MERS-CoV spike protein and developing progressive severe pneumonia with virus detected in the frontal lobes of the brain and in the cerebellum within 6 days after infection [16]. We previously developed a transgenic mouse expressing human DPP4 (hDPP4) and found that these mice develop severe acute respiratory failure and extrapulmonary organ dysfunction when infected with MERS-CoV, the viral protein of which was detected in brain tissue [17].

The adult brain, once considered immunologically privileged, is subject to immune surveillance and possesses its own immune competence. Systemic and local immune responses utilize the complement system [18, 19], the components of which are predominantly synthesized in the liver. However, neurons, astrocytes, microglia, and oligodendrocytes in the brain are also able to synthesize complement proteins [20, 21], which contribute to brain development and homeostasis [22–24]. However, damage in the brain results in rapid or uncontrolled activation of complement proteins, leading to the release of inflammatory anaphylatoxins, such as C3a and C5a, inflammatory cell recruitment, and breakdown of blood brain barrier (BBB) [25, 26]. The presence of MERS-CoV in the brain may induce a similar response.

To understand the neuropathologic effects of coronaviruses such as MERS-CoV, we investigated how MERS-CoV infects the brain. Using a transgenic mouse model, we found that MERS-CoV infects neurons as well as astrocytes, resulting in microglial activation. Infection was accompanied by BBB breakdown and neuronal apoptosis, which were likely aggravated by complement activation. This dysregulation of the inflammatory response in the brain may contribute to the neuropathology of MERS-CoV infection. Thus, the complement system represents a potential therapeutic target for the adjunctive treatment of coronavirus infections.

METHODS

Mice and virus

Six-week-old female hDPP4 transgenic mice [17] were intranasally inoculated with MERS-CoV (HCoV-EMC/2012 strain; 50% tissue culture infectious dose [$TCID_{50}$] of $10^{3.3}$) in 20 µl Dulbecco's modified Eagle's medium. Mice were

Table 1. Distribution of viral antigen by days in hDPP4-Tg mice

DPI	No.	OB	CC	Brain stem	Cerebellum
1	1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–
2	4	±	–	–	–
	5	–	–	–	–
	6	–	–	–	–
3	7	±	–	–	–
	8	–	–	–	–
	9	–	–	–	–
4	10	–	–	+ +	–
	11	–	–	+	–
	12	–	–	+ +	–
5	13	+	+ +	+ + +	–
	14	+	+ + +	+ + +	+
	15	+ +	+ +	+ + +	–

–Negative, ±little, + mild, + +moderate, + + +severe.
CC, Cerebral cortex; DPI, days post-inoculation; OB, Olfactory bulb.

treated intravenously (600 μg kg^{-1}) with a monoclonal antibody (mAb) to the mouse C5a receptor (C5aR1, HM1076; Hycult Biotech, PB Uden, The Netherlands) for complement inhibition immediately after virus challenge or with the same volume of isotype antibody (HI4041, Hycult Biotech, PB Uden, The Netherlands) as a control. Brain tissues were collected on days 1 to 7 after virus challenge for analyses.

Histopathology

Mouse brains were sectioned at a thickness of 4 μm and stained with hematoxylin and eosin (H&E) according to standard procedures for examination by light microscopy. Brain tissue lesions were assessed according to the extent of oedema and hyperemia, the number of red-stained neurons which is the presentation of pyknosis, shrunk neurons and indicative of acute neuronal injury, and the presence of lymphatic sheaths around vessels. The results from all animals in a group were averaged for a total score.

Quantitative RT-PCR

hDPP4 expression was verified by quantitative RT-PCR as previously described [27]. Briefly, total RNA was extracted from different tissues of hDPP4 transgenic mice and undergone reverse-transcription reaction. The resulting cDNA was subjected to quantitative PCR. The relative amount of hDPP4 was determined by normalizing mRNA expression to that of the endogenous control gene GAPDH. The primers specific for hDPP4 were forward 5′ GGAACAGACGATGCAACTG 3′ and reverse 5′ GACTATACAGTTTCAGTCTG 3′. The primers specific for GAPDH were forward 5′ CAATGTGT CCGTCGTGGATCT 3′ and revers GTCCTCAGTGTA GCCCAAGATG.

Immunohistochemistry and immunofluorescence staining

Paraffin-embedded brains were sectioned at a thickness of 4 μm for staining. For immunohistochemistry staining, the sections were incubated overnight at 4 °C with a mouse C3 mAb (Hycult Biotech, PB Uden, The Netherlands), rabbit anti-C3aR or anti-C5aR1 polyclonal antibody (pAb) (Santa Cruz Biotechnology, Dallas, TX), rabbit anti-cleaved caspase-3 pAb (Cell Signalling, Danvers, MA), a neutrophil marker antibody (Santa Cruz Biotechnology), rabbit anti-CD68 polyclonal antibody (Abcam, Cambridge, MA), IBA-1 (FUJIFILM Wako Chemicals, Richmond, VA), or rabbit phospho-p38 MAPK mAb (Cell Signalling). Biotinylated IgG was then added, followed by an avidin-biotin-peroxidase conjugate (Zhongshan Biotechnology, Beijing, China). Immunoreactivity was detected using 3, 3' diaminobenzidine and the sections were counterstained with hematoxylin for observation by microscopy (Eclipse TS100; Nikon, Tokyo, Japan).

For immunofluorescence staining, the sections were incubated overnight at 4 °C with a rabbit pAb to coronavirus (HCoV-EMC/2012 nucleoprotein [NP]; Sino Biological, Beijing, China) or guinea pig pAb against NeuN, IBA-1, or glial fibrillary acidic protein (GFAP). After washing with phosphate-buffered saline (PBS), the sections were incubated with Alexa Fluor 594-conjugated donkey anti-rabbit secondary antibody or Alexa Fluor 488-conjugated goat anti-guinea pig secondary antibody for 2 h at 37 °C. Images were obtained with a laser confocal microscope (LSM710; Zeiss, Germany).

Neutrophil infiltration was assessed using a single representative brain section from three mice within the two experimental groups. The entire area of each section was examined at 400× magnification to obtain an average neutrophil count per field of view. The number of fields of view per brain section were comparable between the two experimental groups.

Intracerebral Evans blue assessment for BBB dysfunction

The extent of BBB dysfunction 7 days after MERS-CoV infection was assessed by Evans blue extravasation in two animals per experimental condition, as previously reported [28]. Briefly, Evans blue (2% in saline, 4 ml kg^{-1}; Sigma) was administered intravenously 2 h before tissue collection. Mice were deeply anesthetized with thiopental sodium and then transcardially perfused with PBS to remove the intravascular dye. Then the brain was collected and photographed.

Viral titres in tissues

Different tissues of infected mice were harvested aseptically on day 7 and homogenized in minimal essential medium containing antibiotics to produce 10% (w/v) suspensions. Tissue homogenates were centrifuged and the supernatants

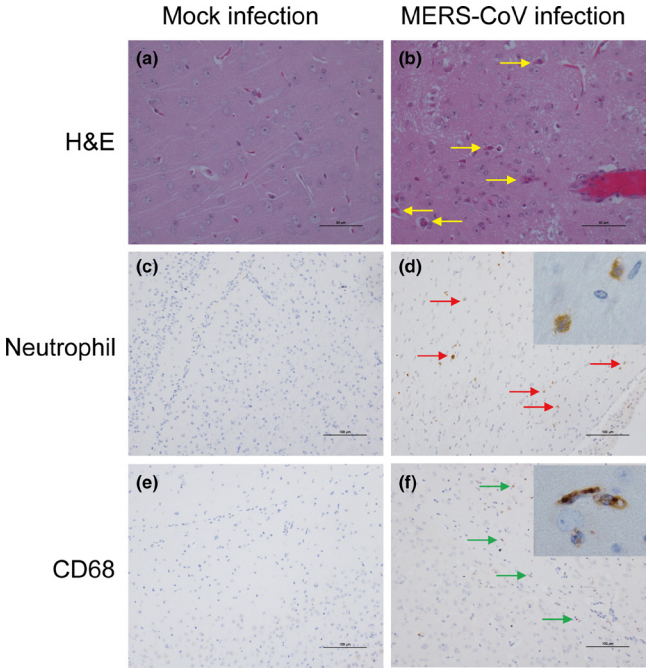

Fig. 2. Brain damage in hDPP4 transgenic mice with MERS-CoV infection. (a, b) Representative images of H&E staining in brain sections of hDPP4 transgenic mice 7 days after infection with MERS-CoV or a mock control (yellow arrows indicate red-stained neurons, neuronal necrosis, and perivascular cuffs). Infiltration of neutrophils (c and d, red arrows) and macrophages (CD68) (e and f, green arrows) was assessed by immunohistochemical staining 7 days after MERS-CoV infection (n=3 per group).

were inoculated to the Vero cells. The cytopathic effects (CPE) were daily observed under phase-contrast microscopy. The viral titres in tissues were calculated using the Reed and Muench method and are expressed as log10 $TCID_{50}$ g^{-1} of tissue.

Statistical analysis

Analyses were performed using GraphPad Prism version 5.01. The H&E scores and the numbers of neutrophils were analysed using Student's *t*-test with Welch's correction.

RESULTS

MERS-CoV infects midbrain-hindbrain tissue in hDPP4-transgenic mice

Although recognized as respiratory pathogens, human coronaviruses are also involved in other pathologies, such as meningitis [4]. To investigate this in an animal model, we utilized hDPP4-transgenic mice, in which lung tissue expresses a higher level of hDPP4 than brain tissue, while only a low level of hDPP4 is expressed in the tissues of kidney, liver, intestine, and spleen (Fig. 1a). Seven days after infection viral titres of MERS-CoV were detectable in lung and brain tissues (Fig. 1b). Neurons and astrocytes but not microglia were infected by the virus, as shown by double immunofluorescence staining of the viral antigen, and the nuclear protein (NP), with NeuN and GFAP, but not IBA-1, respectively, 7 days after infection (Fig. 1c).

Several studies have examined the routes of virus invasion into the CNS [29–31]. Immunohistochemical analyses of brain sections from the transgenic mice revealed that the viral antigen was mainly detectable in brain beginning 4 days after infection (Fig. 1d–i), primarily in the midbrain-hindbrain region (Fig. 1g, l). After 5 days, the viral antigen was detected in the olfactory bulbs and cerebral cortex (Fig. 1j, k). These results are summarized in Table 1.

MERS-CoV infection causes damage to the blood brain barrier and microglial activation in hDPP4-transgenic mice

The BBB is the brain's first line of defence against viral invasion and also plays an important role in maintaining homeostasis. H&E and immunohistochemistry staining revealed the presence of red-stained neurons, neuronal necrosis, and perivascular cuffs (Fig. 2a, b), which were observed in the olfactory bulbs, cerebral cortex, and brain stem (data not shown). In addition, neutrophils and macrophages were detected in the brain parenchyma (Fig. 2c–f), indicating that the BBB may be compromised. Microglia are macrophages of the brain that produce proinflammatory cytokines, such as tumour necrosis factor alpha, interleukin one beta, and nitric oxide, in response to activating stimuli and play a central role in initiating the inflammatory response in the brain [32]. However, excessive activation of microglia results in a neurotoxic inflammatory response [33, 34]. Immunostaining revealed that microglia were activated 4 days after infection, corresponding to the time of detection of the viral antigen in the brain (Table 2, Fig. S1, available in the online version of this article). Thus, microglial activation may be related to the damage induced by MERS-CoV infection.

Inhibition of complement activation reduces the inflammation of brain tissues caused by MERS-CoV infection in hDPP4-transgenic mice

The complement system initiates and amplifies the inflammatory response in various CNS diseases, including Alzheimer's and Parkinson's diseases and multiple sclerosis [35]. However, it is not known if complement activation mediates pathogen-induced neurodegeneration. Immunohistochemical analyses of the brains of hDPP4-transgenic mice infected with MERS-CoV revealed the deposition of complement activation product C3 and higher expression of C3aR and C5aR1 in brain compared with that in mock-infected mice (Fig. S2). These results indicate that MERS-CoV induces excessive complement activation in the brain.

We previously documented severe lung damage in hDPP4-transgenic mice infected with MERS-CoV that was alleviated with complement inhibition [17, 27]. Hemolytic complement activity in normal human cerebrospinal fluid is approximately 0.23% of that in plasma [36]. To clarify the contribution of MERS-CoV-induced complement overactivation to brain

Table 2. Activity of microglia in brain infected with MERS-CoV

DPI	No.	OB	CC	Brain stem	Cerebellum
1	1	–	–	–	–
	2	–	–	–	–
	3	–	–	–	–
2	4	–	–	–	–
	5	–	–	–	–
	6	–	–	–	–
3	7	–	–	–	–
	8	–	–	–	–
	9	+	+	+	–
4	10	+	+	+	–
	11	+	+	++	–
	12	–	–	+	–
5	13	+++	++	+++	+
	14	+++	+++	+++	+
	15	+++	+++	+++	+

–Negative, + mild, + +moderate, + + +severe.
CC, cerebral cortex; DPI, days post-inoculation; OB, olfactory bulb.

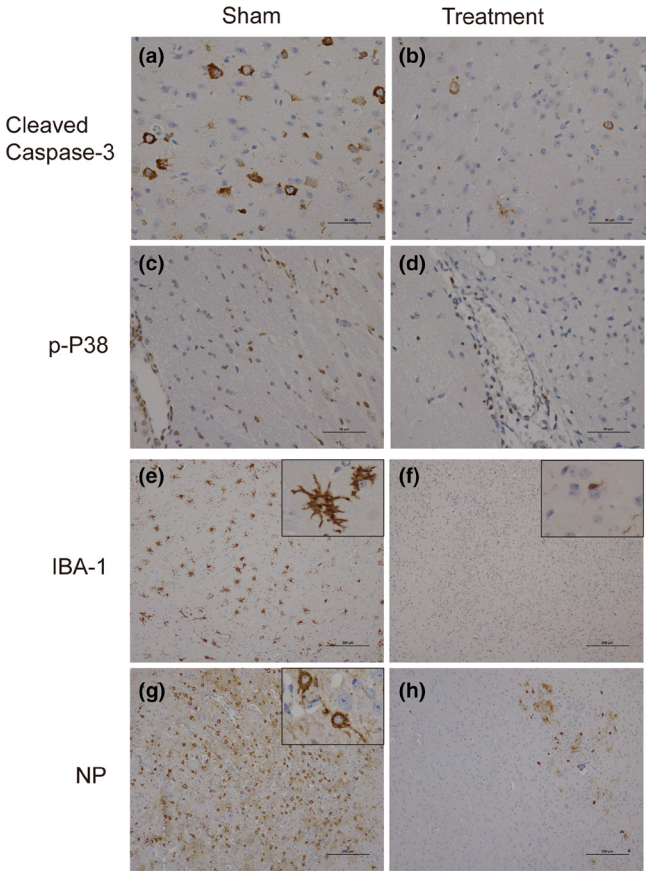

Fig. 3. Anti-C5aR1 antibody treatment decreased inflammation and viral spread in brains of hDPP4-transgenic mice. Representative images of immunohistochemical staining of cleaved caspase-3 (a, b), phosphorylated P38 (c, d), IBA-1 (e, f), and antiviral NP (g, h) in similar regions of cerebral cortex in brains of hDPP4 transgenic mice 7 days after infection with MERS-CoV and treatment with anti-C5aR1 monoclonal antibody. Fewer immunopositive cells were detected in the anti-C5aR1 antibody treatment group (*n*=3 per group).

damage, mice were treated with a mAb to mouse C5aR1 to prevent activation by complement activation product C5a. Mice that were treated exhibited lower expression of activated caspase-3 (Fig. 3a, b), as well as fewer cells expressing phosphorylated p38, than the control group (Fig. 3c, d). Whereas activated microglia were observed throughout the brain 7 days post- MERS-CoV infection, substantially fewer activated microglia were observed in the mice treated with the anti-C5aR1 antibody, and those that were activated had fewer stick-like apophyses (Fig. 3e, f). Viral infection was detected in many neurons of sham-treated mice, whereas NP-positive neurons were only detected in small patches in mice treated with the C5aR1 antibody (Fig. 3g, h). These results suggest that complement inhibition may suppress the neuroinflammatory response and limit viral spread in the brain.

Inhibition of complement activation reduces damage of BBB and brain tissues of hDPP4-transgenic mice with MERS-CoV infection

Mice treated with the anti-C5aR1 antibody also showed less oedema, fewer red-stained neurons, and less inflammatory cell infiltration, especially around vessels (Fig. 4a, b), resulting in lower H&E scores (Fig. 4i). To determine if this protection involved maintenance of the BBB, Evans blue extravasation was assessed in mice 7 days after infection. The brains of MERS-CoV-infected mice appeared blue compared to those treated with anti-C5aR1 mAb (Fig. 4c, d). The infiltration of neutrophils in cortex was reduced, particularly around blood vessels, in mice treated with the antibody when compared to that in control group (Fig. 4e, f and j). The nuclear translocation of NF-κB in endothelial cells in the sham treatment group suggests a possible mechanism for MERS-CoV-induced disruption of the BBB (Fig. 4g, h). Complement activation is implicated in the disruption of the BBB, which may contribute to the severity of brain damage [37]. The results here indicate that MERS-CoV infection may disrupt the BBB, which could be mitigated by blocking activation of the C5aR1 via antibody treatment (Fig. 5).

DISCUSSION

As opportunistic pathogens, human coronaviruses are neuroinvasive and neurotropic to the CNS [4–6, 38, 39], and SARS-CoV induces neuronal death in the absence of encephalitis in transgenic mice expressing human angiotensin-converting enzyme 2 [40]. A case with fatal encephalitis associated with coronavirus OC43 infection was recently reported [41]. Recent cases with neurologic involvement and

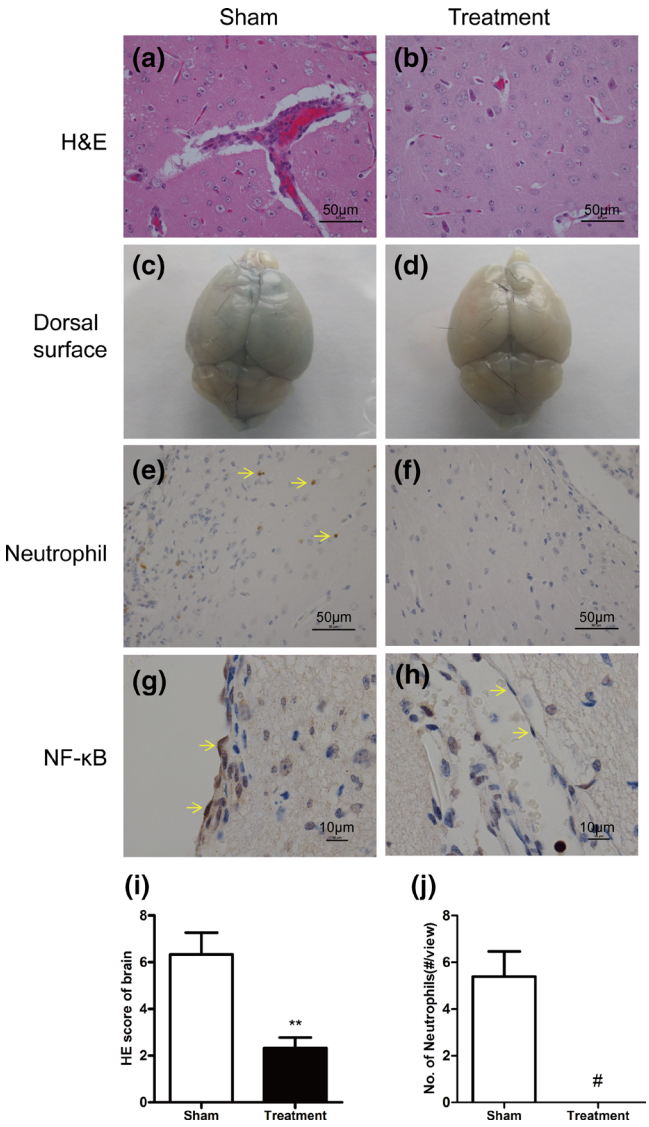

Fig. 4. Anti-C5aR1 antibody treatment decreased brain damage in hDPP4 transgenic mice. (a, b) Representative images of H&E staining of brain sections of hDPP4-transgenic mice 7 days after infection with MERS-CoV and treatment with anti-C5aR1 or sham control. The representative image of the brains in anti-C5aR1 treatment mice showed less oedema, fewer infiltrating inflammatory cells, especially around vessels in the cerebellum compared to those receiving sham treatment. (c, d) Evans blue staining of mice brain on day 7. The brain of a MERS-CoV-infected mouse appeared blue compared with that of a mouse treated with anti-C5aR1 antibody. (e–h) Representative images of immunohistochemical staining for neutrophil infiltration (e, f) and NF-κB localization (g, h). (i, j) Semiquantitative analysis of brain damage *via* H&E scores (i) and neutrophil infiltration (j). #, Undetectable; ***P*<0.01 (Student's *t*-test with Welch's correction)

the detection of viral protein in brain tissues of nonhuman primate and transgenic mouse models indicate that MERS-CoV may also affect the brain [9–12, 16, 17, 42–45]. However, the mechanisms by which the virus invades and damages the brain are not known. The results of the present study indicate that the virus infects the brainstem and induces complement

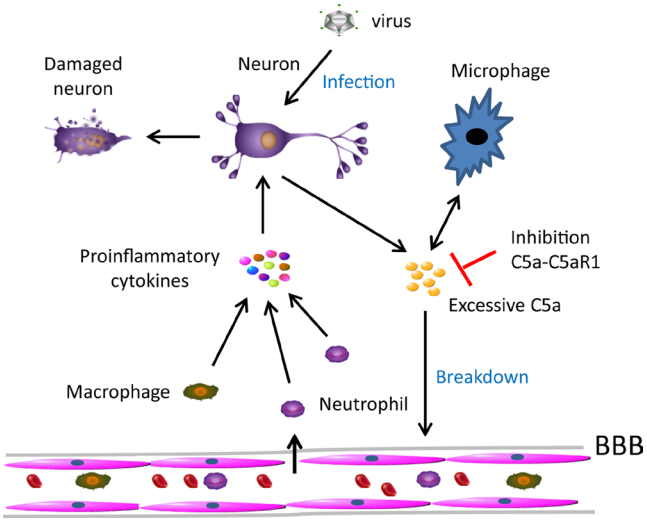

Fig. 5. Diagram illustrating damage to brain tissues in human DPP4-transgenic mice. Neurons infected by MERS-CoV secrete complement components which could activate microglia, which, in turn, could also secrete complement components in brain. Excessive complement activation could activate the endothelial cells of BBB, enhancing the infiltration of inflammatory cells, such as neutrophils and macrophages, into brain parenchyma. The infiltrated inflammatory cells secrete proinflammatory cytokines which could further enhance neuronal damage. However, the inhibition of C5a-C5aR1 interaction could inhibit BBB damage and decrease second damage owing to the excessive inflammatory response.

activation and BBB disruption, thereby may clarify the possible pathogenesis of coronavirus infection-induced encephalitis.

Viral pathogens can invade the brain in several ways, e.g., the hematogenous route, through peripheral nerves, or through olfactory nerves [46]. In our study, mild to moderate viral antigens were detected in the midbrain-hindbrain 4 days after infection, but not in the olfactory bulbs until 5 days after infection, although a little (marked as +/-) viral antigen can also been seen in olfactory bulb of one mouse on two or three d.p.i. (Table 1). So, one possible reason was that MERS-CoV invasion of the brain was somewhat circuitous. The virus initially infected the olfactory neurons but may be cleaned by microglia or antiviral T cells [47] and finally gain the access to the brain via nerves with cell bodies in the midbrain-hindbrain. Once in the brain, the invading virus induces activation of microglia, the resident immune cells that could contribute to neuronal damage in neurodegenerative diseases [32]. MERS-CoV infection resulted in microglia activation in the olfactory bulbs, cerebral cortex, hippocampus, and cerebellum. Overactivated microglia release ROS that cause neurotoxicity [48, 49]. Thus, microglial activation can initiate neuronal loss, as well as amplify ongoing neuronal damage, and may be crucial to the aetiology and the progressive nature of coronavirus infections and other neurodegenerative diseases.

The BBB consists of vascular endothelium and astrocytes as a dynamic interface between peripheral circulation and

the brain parenchyma. The vascular endothelium regulates cellular metabolites and hemostasis, as well as the transport of inflammatory cells to and from the brain [50]. Thus, disruption of this barrier may result in unregulated infiltration of inflammatory cells, leading to secondary damage from viral infection. Cells of the vascular endothelium of the brains of mice in the present study exhibited nuclear translocation of NF-κB after infection with MERS-CoV compared to the control, which may suggest a potential mechanism for BBB dysfunction and subsequent infiltration of neutrophils and macrophages.

Dysregulated complement activation is a pathogenic effector in numerous viral diseases [51]. Complement components in the brain in response to injury can contribute to further brain damage [36, 52, 53]. The brains of mice infected with MERS-CoV exhibited increases in C3 deposition and expression of anaphylatoxin receptors, which suggests that virus-induced complement activation may have contributed to the observed neuropathology. The complement activation product C5a is a potent proinflammatory polypeptide which mediates the strong proinflammatory and immunomodulatory signals in many disease models. It has been studied that C5a activates MAPKs involved in inflammatory cell migration, oxidative bursts from phagocytic cells, and inflammatory cytokine expression [54–58]. In addition, C5a is also a mediator of BBB disruption, which could lead to secondary neuronal disorders and enhance neuronal dysfunction [59–61].

Several monoclonal antibodies, including eculizumab (an anti-C5 mAb) [62], IFX-1 (an anti-C5a mAb) [63] and avdoralimab (an anti-C5aR1 mAb) [64], have been used to block C5a-C5aR1 axis in different studies of COVID-19 infection and results show that antibody treatment exhibits protective effects. Anti-C5aR1 antibody has advantages compared to other two antibodies. It neither restricts membrane attack complex (MAC) formation in the downstream of complement cascade, which is very important in innate immunity, nor influences C5a binding to the other receptor C5L2, which is believed to induce anti-inflammatory response [65]. As an anti-C5aR1 mAb, HM1076 is believed to alleviated brain damage induced by MERS-CoV infection very likely by inhibiting C5a-C5aR1 interaction. However, other mechanisms might be involved considering anti-CD20 mAb has been used to treat B cell malignancies and chronic lymphocytic leukaemia by triggering antibody-dependent cellular cytotoxicity (ADCC) effect [66, 67]. C5aR1 is broadly expressed on the surface of myeloid cells, like neutrophils, monocytes/macrophages, and microglia. One hypothesis is that: these cells were coated with anti-C5aR1 antibody, engaged to natural killer (NK) cells by the bind of antibody Fc region to FcγRIII present on NK cells [68], and finally eliminated by ADCC. This bypath pathway also leads to reduced microglial activation, inflammatory cell infiltration and finally protective effects. To validate this hypothesis, Fc-silent mAb against C5aR1 or mAb that binds C5aR1 but does not block its interaction with C5a might be used as control in the future study.

In summary, the results of this study show that MERS-CoV may infiltrate the brain stem and disperse throughout the brain, resulting in damage. MERS-CoV infection induces complement activation and BBB damage, which is ameliorated by inhibiting C5aR1 activation (Fig. 5). Thus, treatment with a mAb that targets C5aR1 may represent a possible adjunctive therapy for CNS infection by coronaviruses.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The animal studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals and were approved by the IACUC of the Laboratory Animal Center, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology (permit number BIME 2017-0011; Permit Date: 8 March 2017). All experimental operations were performed under sodium pentobarbital anesthesia, and mice were euthanized via inhalation of carbon dioxide.

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