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An Acute Immune Response to Middle East Respiratory Syndrome Coronavirus Replication Contributes to Viral Pathogenicity

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Middle East respiratory syndrome coronavirus (MERS-CoV) was first identified in a human with severe pneumonia in 2012. Since then, infections have been detected in >1500 individuals, with disease severity ranging from asymptomatic to severe, fatal pneumonia. To elucidate the pathogenesis of this virus and investigate mechanisms underlying disease severity variation in the absence of autopsy data, a rhesus macaque and common marmoset model of MERS-CoV disease were analyzed. Rhesus macaques developed mild disease, and common marmosets exhibited moderate to severe, potentially lethal, disease. Both nonhuman primate species exhibited respiratory clinical signs after inoculation, which were more severe and of longer duration in the marmosets, and developed bronchointerstitial pneumonia. In marmosets, the pneumonia was more extensive, with development of severe airway lesions. Quantitative analysis showed significantly higher levels of pulmonary neutrophil infiltration and higher amounts of pulmonary viral antigen in marmosets. Pulmonary expression of the MERS-CoV receptor, dipeptidyl peptidase 4, was similar in marmosets and macaques. These results suggest that increased virus replication and the local immune response to MERS-CoV infection likely play a role in pulmonary pathology severity. Together, the rhesus macaque and common marmoset models of MERS-CoV span the wide range of disease severity reported in MERS-CoV–infected humans, which will aid in investigating MERS-CoV disease pathogenesis. *(Am J Pathol 2016, 186: 630–638; http://dx.doi.org/10.1016/j.ajpath.2015.10.025)*

Middle East respiratory syndrome coronavirus (MERS-CoV) was first isolated in 2012 from a human with fatal acute pneumonia in Saudi Arabia.[1] Since the initial case, >1500 human cases of MERS-CoV infection have been detected (World Health Organization, *http://www.who.int/csr/don/30-september-2015-mers-saudi-arabia/en*, last accessed October 9, 2015); most of these cases have occurred in or near the Arabian Peninsula (Centers for Disease Control and Prevention, *http://www.cdc.gov/coronavirus/mers/about/index.html*, last accessed October 9, 2015). Dromedary camels, common in the Arabian Peninsula, are thought to serve as a reservoir for MERS-CoV,[2] which may, in part, help explain the clustering of human MERS-CoV infections in this geographic location. The exact route of transmission of MERS-CoV from camels to humans has not been definitively identified, although dromedary camels infected with MERS-CoV have been shown to secrete high amounts of infectious virus in their nasal discharge[3] and viral RNA has been detected in their milk.[4]

MERS-CoV causes a wide range of disease severity in infected humans, spanning from asymptomatic to severe,

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fatal pneumonia with acute respiratory distress syndrome occasionally accompanied by acute renal failure or gastrointestinal disease.[5] Most patients present with a fever and respiratory symptoms, which rapidly progress to pneumonia. The most common respiratory symptoms are attributed to lower respiratory tract disease and include dyspnea and coughing.[6] Few individuals solely develop mild upper respiratory tract symptoms, such as a sore throat.[6,7] Severe disease, and death, because of MERS-CoV infection is most common in individuals affected by comorbidities, including diabetes, renal or cardiac disease, and hypertension.[8] The current case fatality rate is approximately 36% (World Health Organization, *http://www.who.int/csr/don/30-september-2015-mers-saudi-arabia/en*, last accessed October 9, 2015); however, no autopsy reports detailing the gross or histological lesions that develop in fatal human infections have been published to date. To elucidate the pathogenesis of this virus and investigate underlying mechanisms for the variation in disease severity seen in humans, two nonhuman primate models of MERS-CoV disease were developed. These models simulated the wide range of disease severity seen in infected humans. After MERS-CoV inoculation, rhesus macaques developed mild to moderate disease, whereas common marmosets exhibited moderate to severe, potentially lethal, disease.[9,10]

Clinical description and virology of MERS-CoV infection in the rhesus macaque and common marmoset models have been reported separately.[9,10] Herein, we focus on detailed and specific histopathology aspects of the respiratory tract of infected animals to better define the pathology of MERS-CoV infection in the lungs. To this end, we quantitatively analyzed the bronchointerstitial pneumonia that developed in both nonhuman primate species after MERS-CoV inoculation and quantified the amount of MERS-CoV antigen in the lungs using digital imaging and analysis. We observed differences in pulmonary neutrophil infiltration and presence of viral antigen in rhesus macaques compared with common marmosets. Increased numbers of neutrophils in the lung and higher amounts of MERS-CoV antigen were observed in marmosets. However, marmosets and macaques had similar pulmonary expression of the MERS-CoV receptor, dipeptidyl peptidase 4 (DPP4). These results suggest that increased pulmonary virus replication and a robust local immune response to MERS-CoV infection may play a role in pulmonary pathology severity, with higher viral loads and a more pronounced acute inflammatory response observed in marmosets.

Materials and Methods

Ethics and Biosafety Statements

All animal experiments were approved by the Rocky Mountain Laboratories (RML; Hamilton, MT) Institutional Animal Care and Use Committee and were performed following the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care, International, by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care, International–approved facility. All infectious work with MERS-CoV was approved by the Institutional Biosafety Committee and performed in a high containment facility at RML. Sample inactivation was performed according to standard operating procedures approved by the Institutional Biosafety Committee for removal of specimens from high containment.

Nonhuman Primates

Archived tissue blocks from eight rhesus macaques (four males and four females; aged 4 to 10 years) inoculated with a total dose of 7×10^6 50% tissue culture infectious dose of MERS-CoV and seven common marmosets (seven males; aged 2 to 6 years) inoculated with a total dose of 5.2×10^6 50% tissue culture infectious dose of MERS-CoV, as described previously,[9–12] were analyzed histologically. The rhesus macaques (RMs 1 to 8) and common marmosets (CMs 1 to 7) were randomly assigned a number. Necropsies of the animals were scheduled for 3 days after inoculation (dpi; CMs 1 to 3 and RMs 1 to 6) and 6 dpi (CMs 4 to 6 and RMs 7 to 8). The remaining common marmoset (CM7) was not originally scheduled for euthanasia; instead, it was to be used to study long-term survival. However, because of development of severe clinical signs, this animal and CM5 were euthanized 4 dpi. A complete set of tissues from each animal was collected at necropsy.

Histopathology and IHC

Histopathology and immunohistochemistry (IHC) were performed on rhesus macaque and common marmoset tissues. Tissues were fixed according to standard operating procedures for a minimum of 7 days in 10% neutral-buffered formalin, embedded in paraffin, and stained with H&E.

IHC with a rabbit polyclonal antiserum against HCoV-EMC/2012 (1:1000; RML)[12] as a primary antibody was used to detect MERS-CoV antigen. IHC was further used to detect neutrophils (polyclonal goat anti-myeloperoxidase, 1:450; R&D Systems, Minneapolis, MN), T cells (monoclonal rabbit anti-CD3, prediluted; Ventana, Tucson, AZ), B cells (polyclonal rabbit anti-CD20, 1:100; Thermo Scientific, Waltham, MA), macrophages (polyclonal rabbit anti-Iba1, 1:1000; RML), epithelial cells (polyclonal rabbit anti-pan cytokeratin, 1:50; Novus Biologicals, Littleton, CO), and DPP4 (polyclonal rabbit anti-DPP4/CD26, 1:100; LifeSpan BioSciences, Inc., Seattle, WA). DPP4 was labeled purple using the Discovery Purple kit (Ventana).

Sections of lung from animals necropsied 3 or 6 dpi that were labeled for MERS-CoV antigen or inflammatory cell markers were digitized using an Aperio Digital Slide Scanner (Leica, Wetzler, Germany) and analyzed using the positive pixel count algorithm in ImageScope version