a noninvasive method to aid in the diagnosis, localization, and assessment of disease activity.

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Emerging Human Middle East Respiratory Syndrome Coronavirus Causes Widespread Infection and Alveolar Damage in Human Lungs

To the Editor:

Middle East respiratory syndrome coronavirus (MERS-CoV) has alerted the public health systems by causing lethal respiratory disease in 54 of 114 confirmed human cases as of September 7, 2013 (1, 2). Coronaviruses represent a diverse family of enveloped, single-stranded positive-sense RNA viruses. In humans, the four endemic coronaviruses HCoV-229E, -OC43, -NL63, and -HKU1 are known to cause mild respiratory symptoms in the majority of cases (3). In contrast, the phylogenetically distinct severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) caused a global outbreak of severe lower respiratory tract disease (4). The clinical symptoms observed in several MERS-CoV–infected humans were related to pneumonia and severe acute lung injury (2) and closely resembled those observed in individuals suffering from SARS (4). Because autopsy studies have not been reported, essential information is missing about the virus- and host-dependent processes underlying MERS-CoV–related lung damage. Herein, we used an *ex vivo* model of human lung tissue infection (5) and a bronchoalveolar lavage (BAL) sample from a patient with MERS-CoV to describe MERS-CoV replication, tropism, dipeptidyl peptidase 4 (DPP4) receptor expression, and virus-related lung tissue damage.

Ex vivo infection of human lung tissue with MERS-CoV followed by spectral confocal microscopy (methods are available in the online supplement) revealed a widespread cellular distribution of viral antigen in alveolar tissue using antisera from infected patients (Figure 1A and Figures E1A and E1B in the online supplement), whereas mock-infected tissue or serum from a healthy donor remained negative (Figures E1A and E1B). As expected for coronavirus, antigen was detected exclusively in the cytosol (Figure E2) (6). Growth curve analyses demonstrated a titer increase of infectious MERS-CoV in the supernatants of infected human lung tissue by more than two orders of magnitude within 48 hours (Figure 1B). This was comparable in extent and kinetics to parallel infections of tissue from the same donors with highly pathogenic avian H5N1 influenza A virus known to cause lethal lung disease in humans (5, 7) (Figure 1B). Double infection of lung specimen with both viruses revealed extensive alveolar infection by MERS-CoV, whereas H5N1 influenza A virus infected only type II cells (5, 7) (Figure 1C).

The investigation of MERS-CoV tropism revealed viral antigen in ciliated bronchial epithelium (Figure 2A) as well as in unciliated cuboid cells of terminal bronchi located in the area of bronchial–alveolar transition (Figure 2B). Lung type I cells comprise approximately 95% of the alveolar surface and therefore form the major area of the lung, being critical for gas exchange (8). Type II cells are crucial for basic lung functions like surfactant production and tissue repair (9). Immunofluorescence (Figure 2C) and electron microscopy (Figures E3 and E4) showed strong MERS-CoV antigen expression and identified intra- and extracellular virions in different stages of the replication cycle

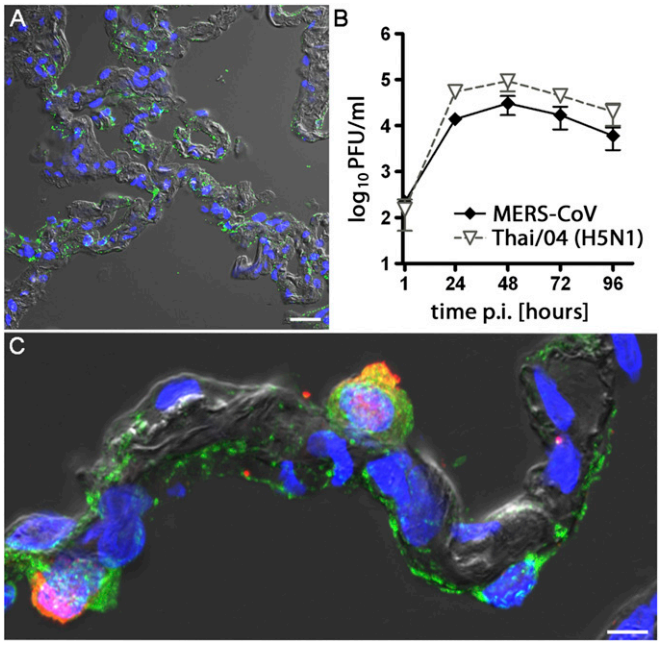

Figure 1. Propagation of Middle East respiratory syndrome coronavirus (MERS-CoV) and detection of viral antigen in *ex vivo* infected human lung tissue. (*A*) Human lung tissue was infected with MERS-CoV for 24 hours and antigen was detected in virus-infected lung tissue using human MERS-CoV antiserum (20) (*green*). Scale bar = 20 μm. (*B*) Human lung explants were infected with either MERS-CoV or H5N1 influenza A/Thailand/1 (Kan-1)/2004 virus (H5N1). Supernatants were collected at the indicated time points and titrated by plaque assay on Madin-Darby canine kidney (MDCK) cells (Thai/04 (H5N1)) or Vero cells (MERS-CoV). Mean values ± SEM of duplicates of four independent experiments are shown. PFU = plaque-forming units; p.i. = post-infection. (*C*) Lung tissue specimens were simultaneously infected with MERS-CoV and Thai/04 (H5N1) for 24 hours. Viral antigens were stained with MERS-CoV antiserum (*green*) and influenza A–specific antibody (*red*). Scale bar = 5 μm. All nuclei (*blue*) were counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

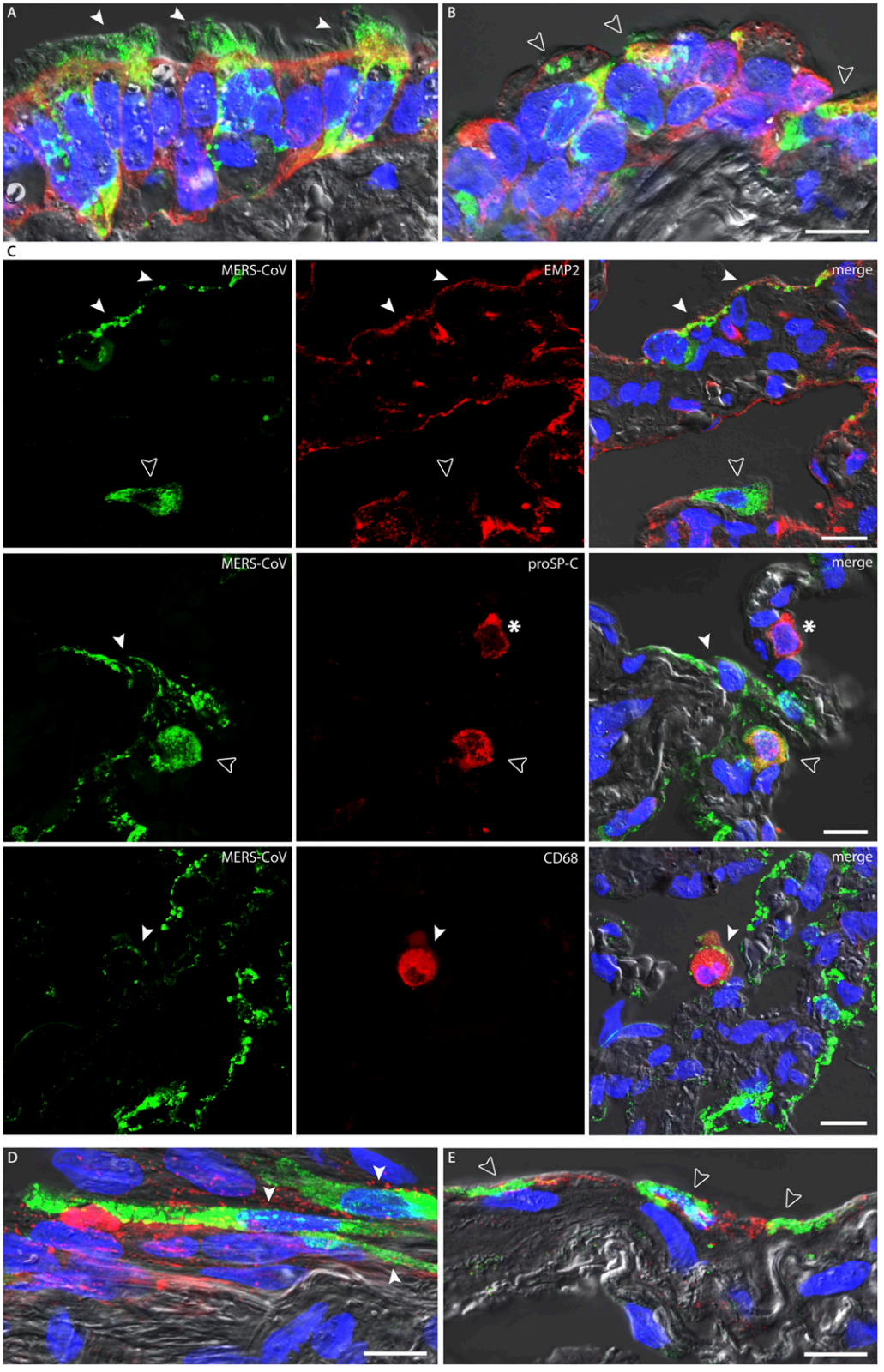

Figure 2. Cellular tropism of Middle East respiratory syndrome coronavirus (MERS-CoV) in *ex vivo* infected human lung tissue. (*A, B*) Histological sections of MERS-CoV–infected tissue samples were probed with MERS-CoV antiserum (*green*) and with antibody against pan-Cytokeratin to confirm epithelial cells (*red*). Infection of ciliated (*white arrowheads*) and nonciliated cells (*open arrowheads*) in simple columnar and simple cuboidal bronchial epithelium is shown. (*C*) Costaining of MERS-CoV with cell markers for type I cells (epithelial membrane protein 2 [EMP2]) (*red*), type II cells (proSP-C) (*red*), or alveolar macrophages (AMs) (CD68) (*red*). MERS-CoV infects type I (*white arrowheads*) and type II (*open arrowheads*) cells but not AMs (*white arrowheads*). The *asterisks* indicate an uninfected type II cell. (*D, E*) Costaining of MERS-CoV antigen (*green*) and von Willebrand factor (*red*) as an endothelial cell marker demonstrates infection of endothelial cells within large (*white arrowheads*) and small (*open arrowheads*) vessels. Scale bars = 10 μm.

in both type I and type II cells. Maximum intensity projection of a typical infected alveolar area (Figure E5) and three-dimensional rendering (Video E1) illustrated the widespread infection of the alveolus. In contrast, less than 1% of alveolar macrophages (AMs) exhibited intracellular viral staining, although viral antigen was frequently detected on the surface of AMs neighboring infected epithelial cells (Figure 2C and data not shown). MERS-CoV antigen was regularly found in endothelial cells of large (Figure 2D) and small pulmonary vessels (Figure 2E). We cannot rule out that MERS-CoV gains direct access to the endothelium via unclosed lung vessels in this *ex vivo* model. However, the virus was detected in urine samples of a patient with MERS-CoV (10), and electron microscopy demonstrated the presence of virus particles in the basal lamina below

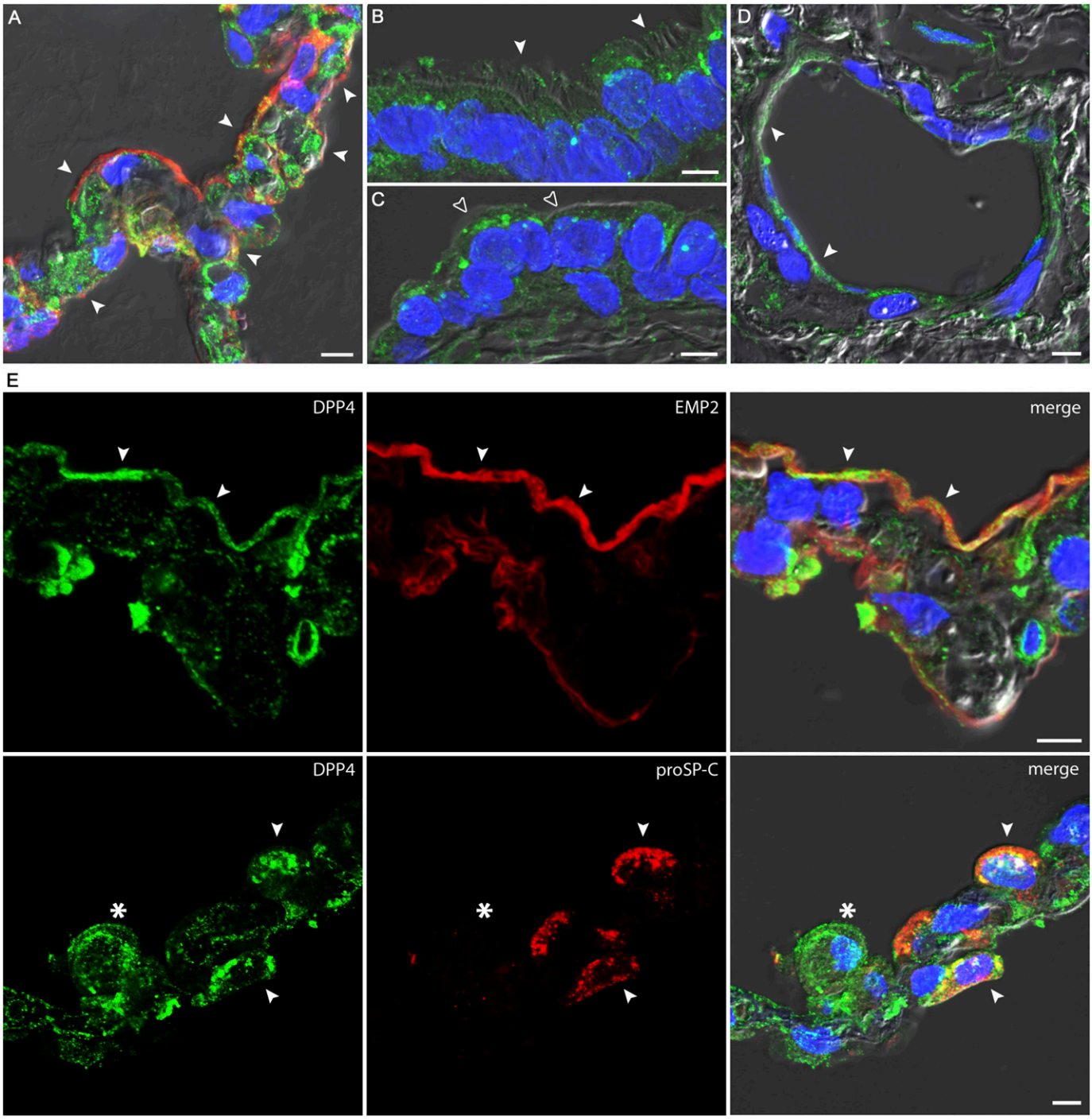

Figure 3. Dipeptidyl peptidase 4 (DPP4) expression in the human lower respiratory tract. (*A*) Histological sections of Middle East respiratory syndrome coronavirus (MERS-CoV)–infected tissue samples were costained for MERS-CoV (*green*) and DPP4 (*red*). MERS-CoV antigen could be detected in DPP4-expressing cells (*white arrowheads*). (*B–D*) In uninfected human lung tissue, DPP4 (*green*) is expressed in (*B*) ciliated (*white arrowheads*) and (*C*) nonciliated bronchial epithelial cells (*open arrowheads*) and (*D*) endothelial cells (*white arrowheads*). (*E*) Tissue sections were stained against DPP4 (*green*) and epithelial membrane protein 2 (EMP2) (*red*) or proSP-C (*red*) as a type I and type II cell markers, respectively. Expression of DPP4 was found in both cell types (*white arrowheads*), as well as alveolar macrophages (*asterisks*). All nuclei (*blue*) were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars = 5 μm.

intact type I pneumocytes (Figure E3D), suggesting basolateral release of the virus. Therefore, it appears a realistic possibility that the virus can enter the bloodstream followed by endothelial infection *in vivo*. Overall, the broad tropism in the human lung indicates that MERS-CoV can infect and replicate in most cell types composing the human alveolar compartment.

The proline exopeptidase DPP4 was recently identified as functional receptor for MERS-CoV as it bound the S1 domain of the viral spike protein and rendered cultured cells of different mammalian species susceptible to the virus (11). However, the role of DPP4 in mediating virus entry in human lung tissue is uncertain, as its expression in the lower respiratory tract has not

been determined. We found a broad expression of DPP4 in MERS-CoV–infected alveolar tissue (Figure 3A). The analysis of mock-infected tissue revealed a constitutive expression in ciliated (Figure 3B) and unciliated (Figure 3C) bronchial epithelium, lung endothelium (Figure 3D), alveolar type I and type II cells (Figure 3E), and AMs (Figure E6), indicating a general role of DPP4 in facilitating virus entry in the human lung.

Damage of the alveolar structure is a hallmark of diseases involving severe respiratory failure (12). Our infection experiments showed detachment of MERS-CoV–infected type II cells from the alveolar base membrane (Figures E7A and E7B). This was accompanied by disruption of alveolar tight junctions (13) in areas with detached infected type II cells visualized by staining of the integral tight junction protein occludin (14) (Figures E7C and E7D and Video E2). Chromatin condensation, nuclear fragmentation, and membrane blebbing of infected type II cells (Figures E7E–E7H) coming off of the alveolar wall pointed to apoptosis (15) of infected cells. In line with these observations, the evaluation of the single available BAL of a hospitalized patient with MERS-CoV showed infected lung epithelial cells with chromatin condensation and nuclear fragmentation as well as hallmarks of apoptosis (Figures E7I–E7L) and the same in infected BAL leukocytes (Figures E7M–E7P). Although the hitherto scarce available material allowed no further in-depth investigation (including leukocyte differentiation), the results indicate that BAL cells of patients with MERS-CoV could be principally useful material for further analysis of MERS-CoV pathogenesis besides using BAL for virus detection.

MERS-CoV continues to cause lethal lower respiratory tract disease (1), raising urgent fundamental questions as to its cellular tropism and receptor usage in alveolar lung tissue, as well as to its pathogenic mechanism(s). In the absence of autopsy data from human victims, we succeeded to model MERS-CoV propagation in human lung tissue and demonstrated an almost pantropic infection, as well as ubiquitous DPP4 receptor expression in bronchiole, alveoli, or vessels. Thus, antiviral approaches that block DPP4 usage are expected to reduce virus propagation in the distal parts of the respiratory tract. We presented first evidence for rapid appearance of structural damage to the alveolar barrier in MERS-CoV–infected tissue, which may significantly influence lung function in several ways: The widespread infection of type I cells may directly reduce oxygen uptake capacity of the lung. Type II cell death reduces surfactant production (16), is expected to diminish the repair capacity of the injured lung (17), and paves the way for alveolar collapse and edema formation, which further impairs gas exchange (9, 12). Deterioration of the alveolar barrier in MERS-CoV–infected individuals may furthermore enable pathogen entry and systemic spread as noticed in SARS-CoV infection (7, 18).

The capability of MERS-CoV to induce alveolar cell death in conjunction with extensive infection of the huge alveolar surface is consistent with acute lung injury observed in MERS-CoV–infected humans (2) and rhesus macaques (19) as well as findings in patients with SARS (7, 18). Additional analysis is required to distinguish whether leukocytes constitute auxiliary targets for the virus or whether the observed antigen staining results from ingestion of infected cell debris. The here-observed capability of MERS-CoV to infect virtually the complete alveolar compartment with morphological correlates of severe lung injury is disconcerting regarding expectable morbidity and mortality. It seems necessary to reach conclusions regarding sources and transmissibility of this emerging virus.

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