INVITED REVIEW

Pathogenesis of Middle East respiratory syndrome coronavirus

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Abstract

Human coronaviruses (CoVs) mostly cause a common cold that is mild and self–limiting. Zoonotic transmission of CoVs such as the recently identified Middle East respiratory syndrome (MERS)–CoV and severe acute respiratory syndrome (SARS)–CoV, on the other hand, may be associated with severe lower respiratory tract infection. This article reviews the clinical and pathological data available on MERS and compares it to SARS. Most importantly, chest radiographs and imaging results of patients with MERS show features that resemble the findings of organizing pneumonia, different from the lesions in SARS patients, which show fibrocellular intra–alveolar organization with a bronchiolitis obliterans organizing pneumonia–like pattern. These findings are in line with differences in the induction of cytopathological changes, induction of host gene responses and sensitivity to the antiviral effect of interferons *in vitro* when comparing both MERS–CoV and SARS–CoV. The challenge will be to translate these findings into an integrated picture of MERS pathogenesis in humans and to develop intervention strategies that will eventually allow the effective control of this newly emerging infectious disease.
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Introduction

Coronaviruses (CoVs) are large, enveloped, positive-sense RNA viruses that infect birds and a wide range of mammals, including humans. These viruses are composed of a few structural proteins that hold a relatively long (around 30 kb) positive-stranded genome (Figure 1). They occur worldwide and can cause diseases of medical and veterinary significance. Generally, infections are localized to the respiratory, enteric and/or nervous systems, although systemic disease has been observed in a number of host species [1,2]. At present, six CoVs have been identified that infect humans. Human CoVs HKU1, NL63, 229E and OC43 predominantly cause a mild respiratory tract infection, characterized by upper respiratory tract disease that includes coryza, cough and sore throat. These viruses only occasionally induce lower respiratory tract disease, including bronchitis, bronchiolitis and pneumonia [2–9]. In contrast, two recently emerged CoVs induce a more severe lower respiratory infection that may be fatal, Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV) [10,11]. The SARS outbreak started in 2002 in China and, after rapid global spread through human-to-human transmission, was halted in 2004. The number of cases reported to the World Health Organization (WHO) was 8096, including 774 deaths [12]. Ten years later, the MERS outbreak started in the Middle East and is still ongoing. A total number of 837 laboratory-confirmed cases have been reported to the WHO, including 291 fatalities [13].

So far, reports describing autopsies of fatal MERS-CoV cases have not been published. Therefore, at this stage one can only speculate about the pathology of MERS-CoV in humans. However, further insight into the pathogenesis and pathological potential of MERS-CoV may be obtained by comparing and contrasting the epidemiology, clinical manifestations and host cell response of MERS-CoV to infection with SARS-CoV, which may also cause a life-threatening lower respiratory tract disease.

MERS–CoV transmission

All human CoVs are thought to originate from animal reservoirs, with SARS-CoV and MERS-CoV being the most recent examples, emerging from bats via masked palm civet cats on Chinese wet-markets and dromedary camels in the Middle East, respectively [2,14–17]. Given the fact that MERS-CoV seems to be widely present in dromedary camels in the Middle East and some parts of Africa [18,19], zoonotic transmission is likely to have originated from this animal species and is expected to continue for a long period of time in these regions. Through usage of a common entry
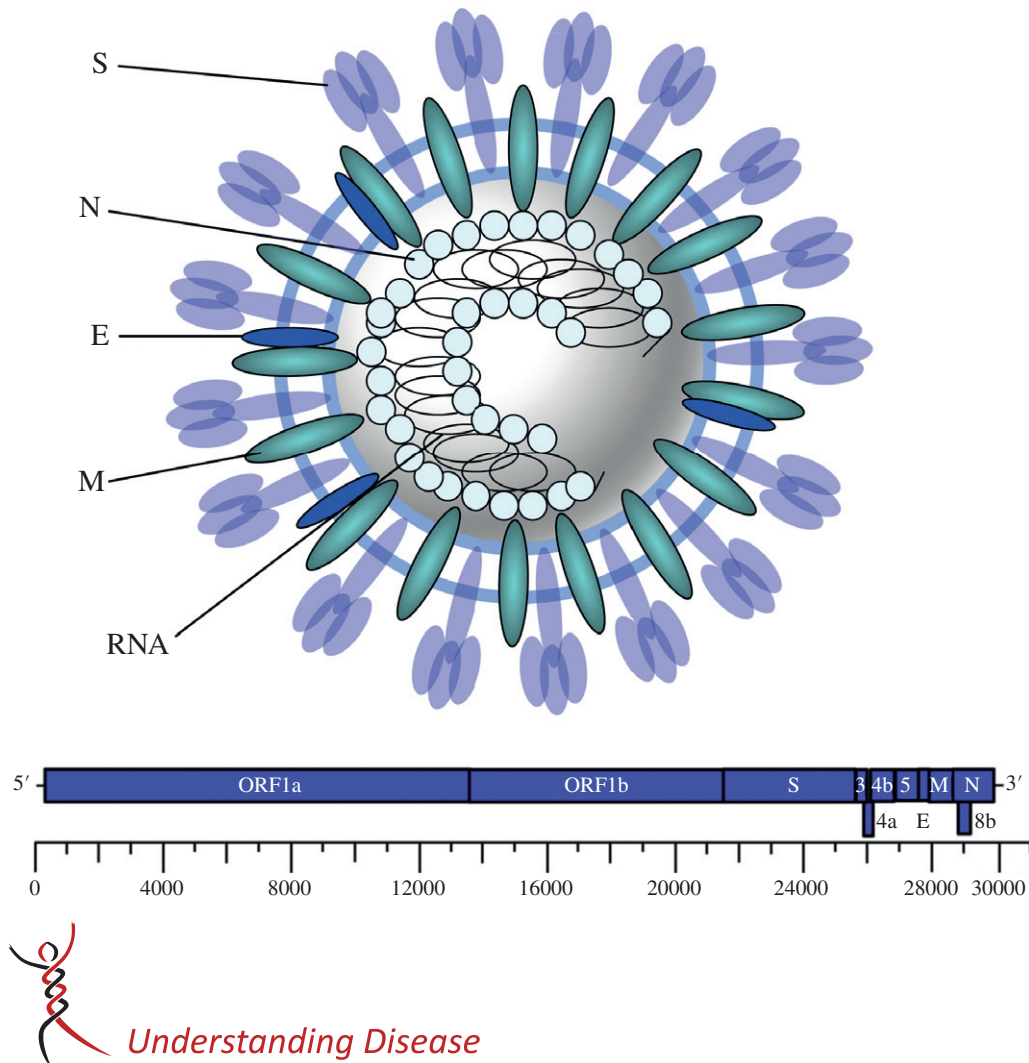
Figure 1. Schematic diagram of a MERS–CoV particle and MERS–CoV genome organization: S, spike protein; M, membrane protein, E, envelope protein; N, nucleocapsid protein.

receptor, dipeptidyl peptidase 4 (DPP4), the emergence of MERS-CoV in humans from dromedary camels, and potentially earlier in time from bats, is facilitated (Figure 2).

Human-to-human spread of MERS-CoV does not seem to be efficient but is reported in hospital outbreaks and travellers returning from the Middle East and their close contacts [20–24]. The hospital outbreaks are mostly due to person-to-person transmission in haemodialysis units, intensive care units or in-patient units, where patients are infected with MERS-CoV of a single monophyletic clade [23]. An outbreak among healthcare workers in a hospital was due to overcrowding and inadequate infection control measures [25]. It is still unclear whether the transmission through person-to-person contact occurs via large respiratory droplets, due to coughing and sneezing, as in SARS, or via fomites [23,25,26]. Also, the episodes of transmission are not clearly defined but are reported to take place during both the symptomatic and the incubation phases [20]. Repeated testing of sputum, nasopharyngeal swabs or bronchoalveolar lavages

(BALs) at different time points will be needed to provide a better understanding [20]. Some of the larger clusters of patients observed in Al Hasa and Jeddah may be primarily related to human-to-human transmission of MERS-CoV, but super-spreader events, as described during the SARS outbreak, have not been noted thus far. This may be due to an overall lower level of virus shedding from the upper respiratory tract contributing to lower transmissibility than seen in SARS [26]. As a result, the outbreak is more restricted when compared to SARS, although future adaptations of MERS-CoV in humans may potentially increase human-to-human transmission or may alter the virulence of the virus, causing more severe disease.

With respect to controlling MERS, rapid isolation and rigorous infection control practices may in the end suffice to limit the outbreak. These include standard contact and airborne precautions, such as wearing a surgical mask, gloves and a gown on entering the room of infected or suspect patients, and removing them on leaving [25]. Dromedary camels infected with MERS-CoV may not show disease but still may excrete MERS-CoV
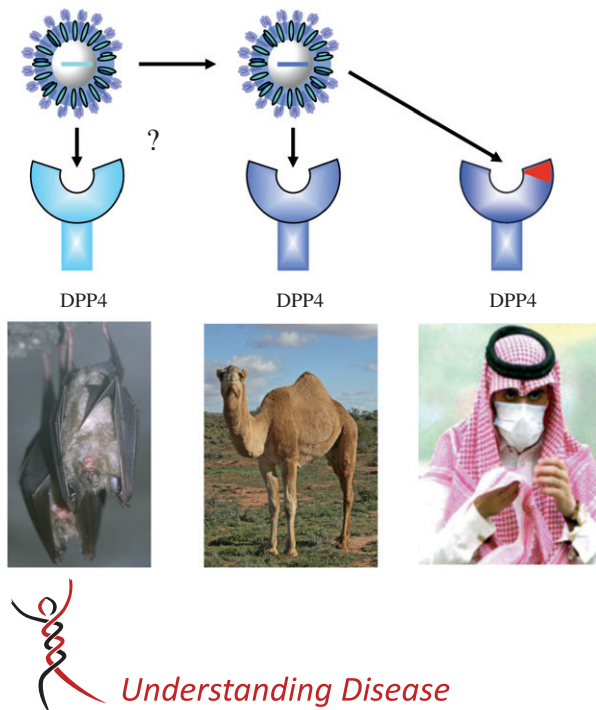
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Figure 2. Zoonotic transmission of MERS-CoV. The emergence of MERS-CoV from dromedary camels is facilitated by the presence of a highly similar viral receptor (DPP4) in humans. Hypothetically, MERS-CoV present in dromedary camels may have emerged from CoVs in bats that also use DPP4 as an entry receptor

through nasal fluids, faeces and, potentially, in their milk and urine [27]. Therefore, the WHO recommends avoiding contact with camels, not drinking raw camel milk or camel urine and not eating meat that has not been thoroughly cooked. Also, people who work, or come into contact with, dromedary camels, such as people working at camel farms, slaughterhouses, markets and camel-racing facilities, and also veterinarians, are at risk and should practise good personal hygiene and wear facial protection and protective clothing where feasible [28].

Clinical aspects of MERS–CoV infection in humans

Most MERS patients acquired the infection in the Middle East, which subsequently led to limited human-to-human transmission in clusters, in healthcare workers and in travel-related cases outside the region, with mild to severe or even fatal respiratory disease. The median incubation period of a MERS-CoV infection is 5 days [20]. Current data indicate that, overall, more men than women have become infected, with a median age of 47 years (range 9 months–94 years), and most fatalities are observed in patients over 60 years [20,22]. Clinical symptoms observed include fever, cough, sore throat, shortness of breath, myalgia, chest pain, malaise and gastro-intestinal symptoms, such as diarrhoea, vomiting and abdominal pain. Less common symptoms

include chills, wheezing, palpitations and confusion [20–22,29]. Respiratory symptoms are mainly related to lower respiratory tract disease (dyspnoea, cough and fever), while upper respiratory tract disease is reported infrequently. A large proportion of the severely ill patients required mechanical ventilation [30]. Interestingly, many of the reported secondary cases showed mild respiratory symptoms or were asymptomatic [31].

Radiology of MERS patients revealed mild to severe pulmonary consolidation. Chest radiographs of a large percentage of the patients admitted to hospital showed airspace and interstitial opacities, with subtle to extensive, unilateral to bilateral, and focal to diffuse distribution. Air space opacities are variable in their distribution, described as reticular or reticulonodular, and demonstrate thickening of bronchovascular areas [20,21,32]. Computed tomography (CT) examination of hospitalized patients with MERS revealed bilateral, mostly subpleural and basilar, airspace involvement, with ground-glass opacities and limited consolidation. The fact that most lesions were found in the subpleural and peribronchovascular region is suggestive of organizing pneumonia [32].

During the course of the infection, MERS-CoV is mainly detected in the lower respiratory tract, while earlier in the infection virus is detected in the upper, as opposed to the lower, respiratory tract [16,26,33–37]. Although virus is detected in urine and blood of some MERS patients, this is not a consistent finding [26,35,36], but indicates that systemic infection can occur. Potential risk factors for the development of severe disease are obesity, diabetes mellitus, end-stage renal disease, cardiac disease, hypertension, lung disease, including asthma and cystic fibrosis, and any immunosuppressive condition [20–23,29,30]. Complications described in fatal cases are hyperkalaemia with associated ventricular tachycardia, disseminated intravascular coagulation leading to cardiac arrest, pericarditis and multi-organ failure [21]. One report demonstrated stillbirth during MERS-CoV infection [38]. When compared to a case-control group, MERS patients were more likely to be admitted to the intensive care unit and had a higher mortality rate [22]. The survival rate in patients detected via the active surveillance system was higher than in the clinically identified cases [23]. Co-infections with other pathogens are also described frequently, but their relevance as a critical factor for disease progression is uncertain [20,29,32].

Laboratory analyses of blood from MERS patients have revealed mild to severe abnormalities. Haematological abnormalities included elevated leukocyte counts and lymphopenia, while a few cases showed lymphocytosis, thrombocytopenia and coagulopathy [20,21,32]. Other laboratory findings included elevated creatinine, lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase levels, suggestive of renal and liver disease or failure [20,21,30,32].

When MERS is compared with SARS, many similarities in the clinical symptoms and respiratory disease become apparent. There are only small differences in

the median incubation period, a slightly longer time for MERS (5.2 days) than for SARS (4 days). In SARS, a wide spectrum of clinical symptoms is described, similar to that seen in MERS; fever, chills, diarrhoea and pneumonia [39]. In both conditions severely ill patients presented with acute hypoxic respiratory failure that often required mechanical ventilation. Although both viruses are considered respiratory viruses, gastro-intestinal infections and symptoms have been described in both SARS and MERS patients. The case-fatality rate may seem higher for MERS-CoV (around 30%) than for SARS-CoV (9.6%), but whether this is the result of a higher virulence of MERS-CoV is not clear, since many MERS-CoV infections may have gone unnoticed. Similar co-morbidities have been described for both MERS and SARS. In MERS when compared to SARS, predominantly diabetes type 2 and chronic renal disease are important co-morbidities [40–47]. In SARS, age is a significant risk factor for the development of severe disease; similarly, for MERS, most fatalities occur in patients aged > 60 years [20,22]. The laboratory findings more often showed higher levels of parameters associated with liver and kidney dysfunction in SARS than in the described cases of MERS, although acute renal failure is often seen in MERS. Complications during pregnancy, such as maternal mortality and stillbirth, have been described in SARS and thus far only rarely seen in MERS cases, but that may be attributable to decreased maternal tolerance to hypoxia and reduced fetal oxygen flow due to respiratory disease [38].

Most importantly, chest radiographs and imaging results of patients with MERS showed opacities and distribution of lesions that resemble the findings of organizing pneumonia seen in patients with pandemic influenza virus A(H1N1)pdm09 [32,48,49]. This is different from the lesions in SARS patients, which show fibrocellular intra-alveolar organization with a bronchiolitis obliterans organizing pneumonia (BOOP)-like pattern [50,51]. Thus, although clinical symptoms may be relatively similar between SARS and MERS patients, the mechanisms leading to disease may actually be different.

Pathology of MERS–CoV infection in humans

So far, no reports describing autopsies of fatal MERS-CoV cases have been published. From the clinical data and X-rays of the severe cases of MERS that have been described in humans, a severe and progressive pneumonia may be suspected, as described for SARS, with diffuse alveolar damage (DAD) in the acute phase and more proliferative change in the later phase of the disease. Pulmonary fibrosis was seen frequently in SARS, including in patients who survived the infection. In the absence of accurate follow-up of MERS patients, there is limited information on the exact course of the disease in the long term. Comparison of the epidemiology, clinical manifestations and host cell response in MERS-CoV infection to infection with SARS-CoV

may provide further insight into the pathogenesis and pathological potential of MERS-CoV.

In SARS patients, gross pathology of the respiratory tract demonstrates variable consolidation, with pulmonary oedema, haemorrhage and congestion, and pleural effusion (as reviewed in [52]). Histopathology of SARS shows DAD with an exudative phase, a proliferative phase and a fibrotic phase. The exudative phase is seen in patients in the initial 10 days of the disease, and is characterized by necrosis of alveolar, bronchiolar and bronchial epithelial cells, intraluminal oedema, fibrin exudation, hyaline membrane formation, haemorrhage and infiltration of inflammatory cells, such as monocytes or macrophages, lymphocytes and neutrophils, into the alveolar wall and lumina [50,53–55]. The proliferative phase, after 10–14 days, shows interstitial and alveolar fibrosis, bronchiolitis obliterans organizing pneumonia (BOOP), regeneration with type II pneumocyte hyperplasia and multinucleated giant cells [50,51,55–57]. The fibrotic phase, after 14 days, shows interstitial thickening, with fibrosis and a BOOP-like pattern and few inflammatory cells (mainly histiocytes and lymphocytes) [51,57]. So far, the clinical data of MERS patients demonstrate a similar disease in MERS. However, there is limited evidence for the development of fibrosis in the end-stage acute respiratory distress syndrome (ARDS) induced by MERS-CoV.

Pathology of experimental MERS–CoV infection in animals

Several animal species, such as rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), marmosets (*Callithrix jacchus*), ferrets (*Mustela putorius*), mice (*Mus muris*), Syrian hamsters (*Mesocricetus auratus*), rabbits (*Oryctolagus cuniculus)*, guinea-pigs (*Cavia porcellus*) and dromedary camels (*Camelus dromedarius*) have been experimentally infected with MERS-CoV to study the pathological changes as a result of the viral infection [58–64]. However, in the absence of any descriptive study on the pathological changes in the lungs of human MERS patients, it is very difficult to interpret data from experimental MERS-CoV animal infection experiments. Overall, the outcome of MERS-CoV infection and the subsequent development of lower respiratory disease seems variable in different animal species inoculated with MERS-CoV.

Rhesus macaques can be infected with MERS-CoV [62] intratracheally or by a combination of inoculation routes [58]. The rhesus macaques developed increased body temperatures at 1–2 days post-infection (dpi) and transient clinical signs, such as increased respiration rate and cough. Haematology showed an increase in total white blood cells and neutrophils from 1–2 dpi and a decrease in lymphocytes from 1–2 dpi [60]. Radiographic imaging revealed localized infiltration and interstitial markings. By gross pathology on 3 dpi, there

was congestion and little indication of acute pneumonia. By histopathology on 3 dpi, there was a multifocal mild-to-moderate interstitial pneumonia characterized by thickened alveolar septa with oedema, fibrin and few macrophages and neutrophils, intraluminal alveolar macrophages, neutrophils and multinucleated giant cells, fibrin and sloughed epithelial cells; perivascular inflammatory infiltrates were present in the interstitium [62]. At 6 dpi there was type II pneumocyte hyperplasia with alveolar oedema, fibrin deposition and hyaline membranes [60]. *In situ* hybridization (ISH) and immunohistochemistry (IHC) demonstrated the presence of virus RNA and antigen expression in type I and II pneumocytes and alveolar macrophages. In rhesus macaques inoculated intratracheally, virus was present in the lungs at 3 dpi, but not in nasal, oropharyngeal or rectal swabs or organs (kidney, trachea, brain, heart, liver, spleen, intestine) by RT–PCR and virus titration [62]. In rhesus macaques with a combined inoculation route, virus was present predominantly in nasal swabs at 1 and 3 dpi, but not in urogenital and rectal swabs. Virus was also present in the bronchoalveolar lavage (BAL) and in the respiratory tract, but not in the kidney. Up-regulation of expression levels of proinflammatory cytokines and chemokines for chemotaxis and neutrophil activation, such as IL-6, CXCL1 and matrix metalloproteinase (MMP)4, was seen in the serum [60]. Similarly, cynomolgus macaques can be infected with MERS-CoV and show virus replication in the lower respiratory tract but, in the lungs, only limited infiltration was observed at different days after infection (van den Brand *et al*, unpublished observations; Figure 3).

Thus, macaque animal models do not fully depict the severe and sometimes fatal pneumonia seen in human patients.

Marmosets infected with a high dose of MERS-CoV [5.2×10^6 median tissue culture infective dose (TCID$_{50}$) via the intratracheal, intranasal, oral and ocular route] develop a more severe disease than rhesus macaques [61]. From 1 dpi the respiration rate increases with loss of appetite, developing into open-mouth breathing and laboured breathing at 3–6 dpi. A few animals had to be euthanized before the end of the experiment. No significant alterations in the blood were demonstrated, except for hypoproteinaemia in the severely ill animals. Radiology by X-rays showed progressive mild to severe interstitial infiltration starting at 1 dpi that was resolved at 13 dpi. Gross pathology showed multifocal consolidation with increased relative lung weights. By histopathology, there was multifocal to coalescing, moderate to marked broncho-interstitial pneumonia centred around the terminal bronchioles and adjacent alveoli.

In other animal species, including dromedary camels that act as the reservoir species, the virus is suggested to induce only limited clinical symptoms. In most cases reported so far, overt clinical disease is absent in dromedary camels positive for MERS-CoV. Dromedary camels from the Middle East, Africa and Spain have been found to harbour MERS-CoV-specific antibodies [16–18,65]. In dromedary camels inoculated intratracheally, intranasally and intraconjunctivally with MERS-CoV, nasal discharge was seen at 2–14 dpi with excretion of virus in the nose, while no virus was found in fecal samples or urine. One dromedary
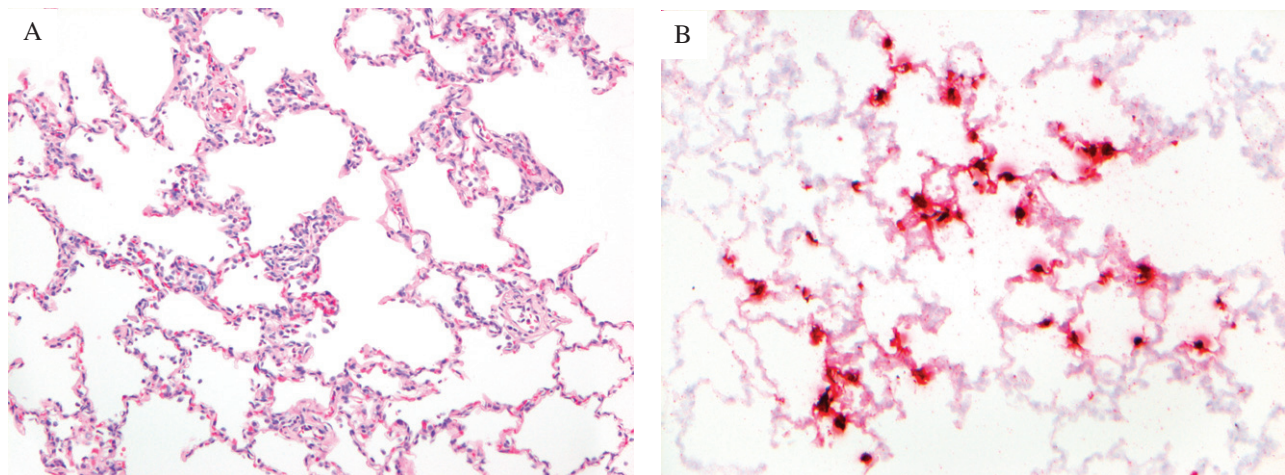

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Figure 3. Histopathology and *in situ* detection of MERS-CoV in the lungs of a cynomolgus macaque after intratracheal inoculation with MERS-CoV. (A) The alveolar septa are mildly thickened, with infiltration of a few neutrophils and monocytes. There are also increased numbers of intraluminal alveolar macrophages and a few neutrophils; H&E, magnification = ×10. (B). MERS-CoV specific RNA by *in situ* hybridization (ISH), targeting the MERS-CoV nucleocapsid, is present in lung tissue of of a cynomolgus macaque after intratracheal inoculation with MERS-CoV. RNA is present predominantly in type II alveolar epithelial cells, with less in type I epithelial cells; ISH, magnification = ×10.

camel at 5 dpi had mild to moderate inflammation and necrosis in the nose, trachea, bronchi and bronchioles, but not in the alveoli. Virus antigen was present, with subsequent presence of infectious virus, in nasal, laryngeal, trachea, bronchial and bronchiolar epithelium, the tonsils and draining lymph nodes, but not in the alveoli [66]. Similar observations have been made in rabbits experimentally infected with MERS-CoV (van den Brand *et al*, unpublished). Virus was excreted from the upper respiratory tract and detected in the lungs, although no clinical signs were observed and there were limited histopathological changes. Ferrets, guinea-pigs and Syrian hamsters cannot be infected; there is no virus replication and no seroconversion [59,62,64].

Since wild-type and innately immune-deficient mice cannot become infected with MERS-CoV [63], an infectable mouse model has been generated by transducing mice with a recombinant non-replicating adenovirus expressing the human host cell receptor DPP4. These mice show widespread hDDP4 expression in airway and alveolar epithelial cells. After MERS-CoV infection, there is no mortality but aged mice lose weight and have abundant virus in the lungs that is cleared by 6–8 dpi. In the lungs there is antigen expression with perivascular and peribronchiolar lymphocytic infiltrates, progressing to interstitial pneumonia. The disease is more severe when there is no type-I IFN signalling, and the T cell response is necessary for virus clearance [67].

Pathogenesis of MERS–CoV

Although MERS and SARS resemble each other clinically, *in vitro* studies have highlighted remarkable differences between these viruses with respect to their growth characteristics, receptor usage and host responses, suggesting that their pathogenesis may be quite different. One way to predict the changes in the lungs after MERS-CoV infection is to use human tissue that has been infected *ex* vivo [68]. Hocke *et al* [69] demonstrated widespread MERS-CoV antigen expression in type I and II alveolar cells, ciliated bronchial epithelium and unciliated cuboid cells of terminal bronchioles, using spectral confocal microscopy. Virus antigen was also found in endothelial cells of pulmonary vessels and rarely in alveolar macrophages. Electron microscopy revealed alveolar epithelial damage, consisting of detachment of type II alveolar epithelial cells and associated disruption of tight junctions, chromatin condensation, nuclear fragmentation and membrane blebbing, the latter suggesting apoptosis [69]. Although this *ex vivo* model does not fully mimic the situation *in vivo*, these changes are in line with observations in cell lines infected with MERS-CoV. Severe cytopathic effects were observed in human hepatoma cells infected with MERS-CoV; these were more severe than those due to SARS-CoV infection [70], although the *in vivo* relevance of this observation remains unclear.

DPP4 (also named CD26) has been identified as the receptor for MERS-CoV [71]. All HCoV receptors identified to date are exopeptidases, although their proteolytic activity is not necessary for the virus to bind to the receptors, nor for them to enter the host cell [69,72,73]. A comparative analysis of HCoV receptor expression across the respiratory tract of humans may provide clues regarding differences in pathobiology between HCoVs. In cell lines and *ex vivo* lung cultures, DPP4 is expressed in type I and II alveolar cells, ciliated and non-ciliated bronchial epithelium, bronchial submucosal glands, endothelium, alveolar macrophages and leukocytes [68,71]. This largely corresponds with viral tropism in *ex vivo* human lung cultures, which show infection of non-ciliated cells in bronchi, bronchioles, endothelial cells and type I and II pneumocytes, but rarely in alveolar macrophages [68,71,75–78]. Remarkably, the binding site of DPP4 is different in different species, explaining why not all animals can be infected with MERS-CoV [64].

SARS-CoV and NL63 use a different receptor for entry into cells, angiotensin-converting enzyme 2 (ACE2) [72,79], which shows in part a similar cell-type tropism as is seen for DPP4. In humans, ACE2 is expressed in ciliated bronchial epithelial cells, type I and II pneumocytes and arterial and venous endothelial cells, but not in tissues of the upper respiratory tract, suggesting that these tissues are not the primary site of entrance for SARS-CoV or NL63 [80,81]. SARS-CoV infects some ACE2-positive cells – ciliated bronchial epithelial cells, bronchioles and type I and II pneumocytes, but not endothelial cells [82].

Comparative genomics provides a way to study the molecular basis for the host response against different but related viral pathogens, as was demonstrated previously in SARS-CoV infection of different primate host species [83–86]. At present, only one comparative *in vitro* study using MERS-CoV has been performed [87]. Calu3 cells, differentiated into polarized ciliated cells, permit replication of both SARS-CoV and MERS-CoV at similar levels. However, MERS-CoV induces a substantial cytopathic effect, starting 18–24 h after infection, whereas SARS-CoV maintains steady replication and cell viability until 72 h after infection [70,77,87]. On the other hand, MERS-CoV seems more sensitive to prophylactic and therapeutic treatment of infection *in vitro* than SARS-CoV [70]. In addition, MERS-CoV induces much greater dysregulation of the host response to infection than SARS-CoV. MERS-CoV specifically down-regulates genes involved in the antigen-presentation pathway, which could have substantial implications for the development of adaptive immune responses [87]. With respect to the analysis of the immune response in humans infected with MERS-CoV, limited data from two patients with different disease outcomes are available to date. In BAL and serum from the patient with a poor outcome, there was a decrease in interferon (IFN)α as well as decreased expression of retinoic inducible-acid gene (*RIG*)-1, melanoma differentiation-associated protein 5

(MDA5) and interferon regulatory factors (IRF)3 and IRF7, which are involved in the recognition of viruses by the innate immune system. In the patient with poor outcome there were also high levels of CXC-motif chemokines ligand (CXCL)10 and interleukin (IL)-10, which may have resulted in lower IFNγ expression and higher levels of IL-17A and IL-23 [88]. In the patient who survived, rapid clearance of the virus with increased levels of IL-12 and IFNγ was noted [88].

In SARS, the development of severe lower respiratory tract disease correlates partly with aberrant immune responses, with unbalanced cytokine and chemokine profiles [39,50,89,90]. The levels of both cytokines and chemokines in the blood are elevated: IL-1, IL-6, IL-8, IL-12, IFNγ, monocyte chemotactic protein (MCP)-1 (or CC-motif ligand 2, CCL2), monokine induced by IFNγ (MIG), IFN-inducible protein (IP-10, or CXCL10), and transforming growth factor (TGF)β [89–94]. Some of these chemokines are important for chemotaxis and activation of neutrophils and mono-cytes [95–97], which corresponds with the infiltration of these cells in the respiratory tract of human SARS cases [83,91,92,98]. It remains to be determined which host responses dominate MERS-CoV infection *in vivo* but, based on the results obtained thus far, MERS-CoV and SARS-CoV may induce different pathways.

Conclusion

At this time it is difficult to describe or predict the pathology of severe respiratory disease from MERS-CoV infection. First, there are no reports on autopsies of human fatal MERS cases. This is partly related to the religious backgrounds of the patients, which exclude autopsy. Although limited numbers of human fatal SARS patients have been described, these studies indicate an immunopathological component that may dominate the pathogenesis of SARS. Second, to gain more knowledge of fatal MERS in humans, further studies are needed in animal models that could provide information on replication dynamics, clinical disease, histological lesions and cellular tropism. Third, the patients with severe MERS have very diverse pre-existing conditions, and therefore it may be difficult to obtain definite answers from these studies. Virus-induced histological changes may be obscured by clinical treatments or pre-existing disease. Thus, it may be very difficult to know the full scope of this disease, including the histological lesions, viral cellular tropism and pathogenesis of MERS-CoV. On the other hand, although there are better descriptive studies of SARS regarding the pathology induced in the lower respiratory tract, these provided limited benefit for the development of intervention strategies. Therapeutically, corticosteroids were used for the treatment of SARS, producing variable improvement with less fever, reduced inflammatory infiltrates and better oxygenation [99]. More promising results were obtained with the administration of IFNs in SARS patients. In fact, *in* *vitro* studies have demonstrated that MERS-CoV is more sensitive to type I IFN than SARS-CoV. Overall, the current available data on MERS-CoV suggest that patients at risk for severe exacerbations after infection are elderly patients or patients who have underlying co-morbid conditions. These patients may not be able to combat the viral infection with type I IFN host responses as efficiently as other patient groups, as aging and co-morbid conditions have been described to have a negative effect on the ability to mount strong type I IFN responses [84,100]. The sensitivity of MERS-CoV *in vitro* to type I IFN may indicate that type I IFN can be used as a prophylactic/therapeutic intervention strategy *in vivo*.

In general, human CoV infections cause a relatively mild respiratory disease in children and many healthy adults. Severe exacerbations of CoV disease, with lower respiratory tract involvement, seem to occur in the elderly and/or patients with underlying co-morbidities. Possibly, the lack of pre-existing immunity against SARS-CoV and MERS-CoV in the human population resulted in a higher infection rate in adults and the elderly during the SARS outbreak than normally observed for the other non-SARS human CoVs, leading to a higher morbidity and mortality. Alternatively, viral factors, including those encoded by different accessory viral genes, may play a decisive role in determining pathogenesis.

Overall, there are a number of potential key *in vivo* and *in vitro* differences between MERS-CoV and SARS-CoV infections. These include radiographic differences suggesting a different type of pneumonia, a large difference in cytopathic effect, differences in host response to infection, and differences in sensitivity to type I IFN *in vitro*. These observations point to different disease-causing mechanisms and warrant further studies into the effect of these two viruses on different cell populations *in vitro*, *ex vivo* and *in vivo*, the outcome of which may have significant implications for the development of intervention strategies.

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Author contributions

All authors contributed to the writing and editing of the manuscript and the design of the figures.

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