OPEN ACCESS Freely available online

PLOS | PATHOGENS

Pearls

Emergence of the Middle East Respiratory Syndrome Coronavirus

Christopher M. Coleman, Matthew B. Frieman*

Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, United States of America

Introduction

It began routinely enough. A patient with severe respiratory disease at the Dr. Soliman Fakeeh Hospital in Jeddah, Saudi Arabia was getting worse and no one knew why. A sample of sputum was sent to Dr. Ali Mohamed Zaki to identify the culprit, as he had identified these diseases many times before. However, this time would be different. The sample showed no positive hits on any of the virus assays he normally used. He contacted Dr. Ron Fouchier, at Erasmus Medical College in Rotterdam, Netherlands, to see if he could be of help. Dr. Zaki's initial idea was that the virus was a paramyxovirus, and Dr Fouchier had recently published a Pan-paramyxovirus polymerase chain reaction (PCR) assay [1]. In Dr. Fouchier's lab, the virus was identified as a novel coronavirus, one that had never been seen before.

This novel coronavirus, now called Middle East respiratory syndrome coronavirus (MERS-CoV), has been identified in several countries across the Middle East and Europe, with primary infections found in Saudi Arabia, Qatar, Jordan, and The United Arab Emirates (UAE) (http://www.who.int/csr/disease/coronavirus_infections/en/). Infections in the United Kingdom, Tunisia, France, Italy, and Germany have been imported by travel from the Middle East. The Italian cluster is believed to be from a patient traveling to Jordan and back, and the French cluster originated from a patient traveling to the UAE. The largest cluster of cases, 23 in total, is in Saudi Arabia. As of July 25, 2013, there are 90 confirmed infections, of which 45 have resulted in death, resulting in a 50% case fatality rate. MERS-CoV has been sequenced from nine infected individuals, and its genome sequence places it in the same sub-family (Group 2) as SARS coronavirus (SARS-CoV), but in a new lineage (called Group 2c) (sequences reported in [2–4] and at http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317138176202; http://www.ncbi.nlm.nih.gov/nuccore/KC776174)(http://www.virology-bonn.de/index.php?id = 46).

What Is the Name of This Novel Coronavirus?

The initial name of this novel coronavirus was hCoV-EMC, which stood for human coronavirus–Erasmus Medical College, where the first isolate was sequenced [3]. An additional isolate, provisionally named human coronavirus England 1, was isolated from a patient in London, UK, who had been flown from Qatar to London for treatment [4]. A report from the Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV) has proposed naming this virus Middle East respiratory syndrome coronavirus (MERS-CoV) [5]. MERS-CoV is provisional until ratified by the ICTV.

Where Did MERS-CoV Come From? Is There a Natural Reservoir?

The closest phylogenetic neighbors to MERS-CoV are putative bat coronaviruses in China (BtCoV-HKU4 and BtCoV-HKU5) [3], the Netherlands (BtCoV/VM314/2008} [2], and a recently discovered isolate from South Africa [6]. All four of these bat coronaviruses have been sequenced only from bat samples and have never been isolated as live viruses from either bats or the environment. The natural reservoir of MERS-CoV has not been identified, although its similarity to these other four viruses suggests that it is of bat origin. Importantly, SARS-CoV emerged from bats as well [7]. Anecdotal evidence suggests that MERS-CoV may have been transmitted to humans via livestock (camels or goats); however, there is no scientific data yet to support this theory.

Does MERS-CoV Share Any Features with SARS-CoV beyond the Zoonotic Origin?

Given the similarities in emergence and apparent zoonotic origins between MERS-CoV and SARS-CoV, initial experiments on MERS-CoV focused on direct comparison with the known molecular biology of SARS-CoV. Infection experiments in cell culture showed that MERS-CoV does not use the SARS-CoV receptor, angiotensin converting enzyme 2 (ACE2), for entry, and that MERS-CoV has a much broader host range than the epidemic isolate of SARS-CoV [8–14]. The genome structure of MERS-CoV is similar to other coronaviruses, with the 5′ two-thirds of the genome encoding the non-structural proteins (NSPs) required for viral genome replication, the remaining 3′ third of the genome encoding the structural genes that make up the virion (spike, envelope, membrane, and nucleocapsid proteins), and four accessory genes interspersed within the structural gene region [2]. One additional similarity between MERS-CoV and SARS-CoV is their abilities to inhibit a robust type I interferon (IFN) response in infected cells. However, MERS-CoV has been shown to be much more sensitive to exogenous type I IFN treatment compared to SARS-CoV, which may be important for pathogenesis [8,11,14,15]. Several SARS-CoV-encoded proteins have demonstrated innate immune signaling antagonism [16], and MERS-CoV encodes

Citation: Coleman CM, Frieman MB (2013) Emergence of the Middle East Respiratory Syndrome Coronavirus. PLoS Pathog 9(9): e1003595. doi:10.1371/journal.ppat.1003595

Editor: Vincent Racaniello, Columbia University, United States of America

Published September 5, 2013

Funding: MBF and CC are funded by R01AI095569. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: mfrieman@som.umaryland.edu

several IFN antagonists as well (Matthews et al, submitted, Muller et al, submitted).

What Is the Receptor for MERS-CoV and What Cells Does It Infect?

MERS-CoV has been shown to infect a range of human, primate, porcine, and bat cell lines [11]. *Ex-vivo* infections of human lungs and human airway epithelial cell cultures identified type II alveolar cells and non-ciliated lung epithelial cells (Clara cells) as the targets of infection, rather than the ACE2-expressing ciliated epithelial cells that SARS-CoV targets [9,15]. Interestingly, in at least one case, endothelial cells were infected as well [15], showing a distinct difference between the biology of SARS-CoV and MERS-CoV, as SARS-CoV specifically infects ciliated epithelial cells in the lung [17,18]. The receptor for MERS-CoV was recently identified as dipeptidyl peptidase 4 (DDP4) by mass spectrometry analysis of Huh7 cell protein bound to the MERS-CoV Spike protein *in vitro* [10]. Transfection and localization experiments demonstrated that DPP4 is indeed the receptor for MERS-CoV and is necessary for infection of a non-permissive cell line [10]. DPP4 has many diverse functions in glucose homeostasis, T-cell activation, neurotransmitter function, and modulation of cardiac signaling [19]. ACE2 does not require enzymatic function in order to act as a receptor for SARS-CoV entry, but the enzymatic function of ACE2 has been linked to severity of the disease following SARS-CoV infection [20]. Similarly, inhibition of the enzymatic function of DPP4 did not affect virus entry *in vitro*; however, the role of DDP4 enzymatic activity has not been investigated *in vivo* [10].

What Is the Host Response to MERS-CoV?

Transcriptional analysis of MERS-CoV infected cells has identified several pathways specifically modulated during infection [9]. MERS-CoV is shown to modulate the innate immune response, antigen presentation, mitogen-activated protein kinase (MAPK), and apoptosis pathways. Inhibition of the MAPK pathway showed reduction in viral replication in culture, pointing to potential therapeutics. Importantly, several studies show that MERS-CoV, similar to SARS-CoV, does not induce an early type I IFN response, suggesting that MERS-CoV may encode proteins that inhibit sensing of the viral RNA during infection [8,11,14,15]. The modulation of these pathways may explain the increased lethality of MERS-CoV.

Is There a Small Animal Model of MERS-CoV for the Study of Pathogenesis?

There is currently no small animal model for MERS-CoV. Rhesus macaques infected with MERS-CoV display pneumonia, reduced appetite, significant lung pathology, and inflammatory infiltrates [21]. However, MERS-CoV does not replicate in BALB/c, C57B/6, 129SvEv, or STAT1 knockout mice on the 129SvEv background (Coleman et al, submitted). Interestingly, mouse DPP4 is highly similar to the human DPP4, varying at only 62 positions out of 767 amino acids residues total (92% similarity). However, the differences tend to be on surface-exposed residues which, therefore, could affect binding of viral spike protein to mouse DPP4. Future structural and functional interaction experiments are needed to identify if the mouse DPP4 interacts differently with MERS-CoV spike, as compared to human DPP4, and if the known mutations allowing for this binding could be used for the development of a mouse model of MERS-CoV.

Are There Approved Treatments or Vaccinations for MERS?

There is no current treatment or vaccination available for MERS-CoV, but, with the continuation of the outbreak, identification of therapeutics is a top priority. Several manuscripts have demonstrated that a variety of therapeutics inhibit MERS-CoV replication in cell culture [9,15]. None have been tested *in vivo*, in part due to the lack of a small animal model, as described above. One promising avenue is to use the knowledge of SARS-CoV and compare it to MERS-CoV. IFNα was shown in multiple models to protect against SARS-CoV-induced disease. MERS-CoV is also sensitive to IFNβ treatment *in vitro* [15]. Ribavirin, a known inhibitor of RNA viruses, has also been demonstrated to inhibit MERS-CoV replication, and together they can inhibit MERS-CoV at nanomolar levels [22]. Other inhibitors were shown to affect specific pathways, specifically the MAPK pathway. The MAPK inhibitor SB203580 was shown to inhibit MERS-CoV replication in VerE6 cells [9]. Additional therapeutics and vaccinations are in development, with a focus on FDA compounds already in use.

Conclusion and Questions

Many unanswered questions remain on this newly identified virus:

1. What is the environmental reservoir of MERS-CoV? Is it transmitted from bats to camels, goats, or cats? Is the virus linked to date palm harvesting? How did it spread to people from the environment?
2. Are there associated comorbidities that predispose someone to contracting MERS? With the age of infected patients skewed toward older males, is there a genetic link to infection? Are the patients generally immunosuppressed?
3. What is the seroprevalence of MERS-CoV in the general population? Has MERS-CoV been circulating for many years between animals and people and only now mutated enough to be able to cause disease in people? Or is this a new spillover event that has not been seen by humans until now?
4. Why doesn't MERS-CoV grow in mouse cells or cause disease in mice? Is it because the viral spike protein doesn't bind mouse DPP4 at all, is it because there are other host factors necessary for entry and replication in mouse cells, or is it due to location and amounts of receptor expression?
5. How do the MERS-CoV proteins contribute to disease? Are there any specific functions of the proteins that allow for enhanced pathogenesis?
6. Since this virus is similar to bat coronaviruses identified in China, Africa, and Europe, why haven't other bat coronaviruses spilled over into people, causing serious disease (with the exception of SARS-CoV [7] and, potentially, hCoV-229E [23])? What is it about MERS-CoV and the conditions in the Middle East that have contributed to viral infection and the high mortality rate?

With the spread of MERS-CoV through the Middle East, one thing is certain at this point: The emergence of the novel SARS coronavirus in 2003 from a zoonotic source in China and its spread around the world is not an isolated incident of coronavirus spread. Continued spillover events will occur from animals to humans in the future. The sooner we understand these current microbial threats, the more people we can save from infection and possible death. If we can identify these microbes in our

environment before they infect us, we can better protect ourselves against future infections.

References

1. van Boheemen S, Bestebroer TM, Verhagen JH, Osterhaus AD, Pas SD, et al. (2012) A family-wide RT-PCR assay for detection of paramyxoviruses and application to a large-scale surveillance study. PLoS ONE 7: e34961. doi:10.1371/journal.pone.0034961
2. van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, et al. (2012) Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. MBio 3(6): e00473–12. doi: 10.1128/mBio.00473-12.
3. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367: 1814–1820.
4. Cotten M, Lam TT, Watson SJ, Palser AL, Petrova V, et al. (2013) Full-Genome Deep Sequencing and Phylogenetic Analysis of Novel Human Betacoronavirus. Emerging Infect Dis 19: 736–42B. doi:10.3201/eid1905.130057.
5. de Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, et al. (2013) Middle East Respiratory Syndrome Coronavirus (MERS-CoV); Announcement of the Coronavirus Study Group. J Virol 87:7790–7792.
6. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, et al. (2013) Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa [letter]. Emerg Infect Dis. Available: http://wwwnc.cdc.gov/eid/article/19/10/13-0946_article.htm#suggestedcitation.
7. Li W, Shi Z, Yu M, Ren W, Smith C, et al. (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310:676–679.
8. Kindler E, Jónsdóttir HR, Muth D, Hamming OJ, Hartmann R, et al. (2013) Efficient Replication of the Novel Human Betacoronavirus EMC on Primary Human Epithelium Highlights Its Zoonotic Potential. mBio 4(1): e00611–12.
9. Josset L, Menachery VD, Gralinski LE, Agnihothram S, Sova P, et al. (2013) Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. MBio 4(3):e00165–13.
10. Raj VS, Mou H, Smits SL, Dekkers DH, Muller MA, et al. (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495(7440):251–4. doi:10.1038/nature12005.
11. Zielecki F, Weber M, Eickmann M, Spiegelberg L, Zaki AM, et al. (2013) Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to SARS-coronavirus. J Virol 87:5300–5304.
12. Gierer S, Bertram S, Kaup F, Wrensch F, Heurich A, et al. (2013) The spike-protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2 and is targeted by neutralizing antibodies. J Virol 87:5502–5511.
13. Jiang S, Lu L, Du L, Debnath AK (2013) Putative conformations of the receptor-binding domain in S protein of hCoV-EMC in complex with its receptor dipeptidyl peptidase-4. J Infect 67:156–158.
14. Muller MA, Raj VS, Muth D, Meyer B, Kallies S, et al. (2012) Human coronavirus EMC does not require the SARS-coronavirus receptor and maintains broad replicative capability in mammalian cell lines. MBio 3(6) e00515–12.
15. Chan RWY, Chan MCW, Agnihothram S, Chan LLY, Kuok DIT, et al. (2013) Tropism and innate immune responses of the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. J Virol 87:6604–6614.
16. Frieman M, Heise M, Baric R (2008) SARS coronavirus and innate immunity. Virus Res 133: 101–112.
17. Kuba K, Imai Y, Rao S, Gao H, Guo F, et al. (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 11: 875–879.
18. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, et al. (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426: 450–454.
19. Hildebrandt M, Reutter W, Arck P, Rose M, Klapp BF (2000) A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defence. Clin Sci (Lond) 99: 93–104.
20. Imai Y, Kuba K, Rao S, Huan Y, Guo F, et al. (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436: 112–116.
21. Munster VJ, de Wit E, Feldmann H (2013) Pneumonia from Human Coronavirus in a Macaque Model. N Engl J Med 368:1560–1562.
22. Falzarano D, de Wit E, Martellaro C, Callison J, Munster VJ, et al. (2013) Inhibition of novel β coronavirus replication by a combination of interferon-α2b and ribavirin. Sci Rep 3: 1686. doi:10.1038/srep01686.
23. Pfefferle S, Oppong S, Drexler JF, Gloza-Rausch F, Ipsen A, et al. (2009) Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. Emerg Infect Dis 15: 1377–1384.