

A New Virulent Human Coronavirus: How Much Does Tissue Culture Tropism Tell Us?

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(See the major article by Fuk-Woo Chan et al on pages 1743–52.)

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In 2002 and 2003, the large and quite frightening worldwide severe acute respiratory syndrome (SARS) epidemic first came, and then went, controlled through old-fashioned containment procedures—pathogen identification, case definition, quarantine, and hospital and community infection control—and through major public health efforts by the World Health Organization (WHO) and national programs in Canada and several countries in Asia, particularly China. The last human cases of this potentially disastrous epidemic occurred in August 2003, and since that time many people have been wondering what is next. In fact, there were 4 small outbreaks of SARS in China following the last cases of the major epidemic, of which 3 originated in laboratories working with the virus and 1 probably occurred because of animal contact [1, 2]. The last evidence of human SARS coronavirus (SARS-CoV)

infection was in April 2004. Thus, SARS seems to be gone, perhaps for good. One of the most exciting consequences of the epidemic has been an explosion of information about CoVs, with an extensive exploration of the biology of the SARS-CoV, as well as the discovery of 2 new human CoV species, NL63 and HKU1. Both of these latter viruses are relatively mild respiratory pathogens, infect commonly individuals of all ages, and behave more like the original human CoVs 229E and OC43 than like SARS-CoV.

There is a general agreement now that SARS-CoV probably originated from a virus of bats, jumped the species barrier into ≥ 1 of several animal species used for exotic meat in China (most likely the palm civet) that were captured in the wild, bred in captivity, and sold in markets, and moved from there to human beings [3, 4]. In 2012 a new, highly virulent human CoV, HCoV-EMC (where “EMC” denotes Erasmus Medical Center in Rotterdam, the Netherlands) emerged in the Middle East [5]. This virus, which is the subject of the report by Chan et al in this issue of the *Journal*, possesses some characteristics similar to those of the SARS-CoV, including an apparently similar or even greater pathogenicity. HCoV-EMC was grown from autopsy specimens obtained from the first reported case, a man living in Jeddah, Saudi Arabia, who, in June 2012, developed severe pneumonia with renal

failure and died. There have now been 10 other proven cases of HCoV-EMC infection. All but 1 case originated in the Middle East (4 in Saudi Arabia, 2 in Qatar, 2 in Jordan, and 1 in either Saudi Arabia or Pakistan), and several of occurred in 3 small clusters, all yielding, by polymerase chain reaction (PCR), viruses almost identical to HCoV-EMC. In a recent cluster, 2 cases have been hospitalized in Birmingham, United Kingdom; the first patient to become ill had been traveling in Saudi Arabia and Pakistan, and the second was a family member of the first and had not traveled outside England [14]. Of the 11 proven cases, 5 have been fatal. The 2 definite cases in Jordan were part of a cluster of 11 clinical cases in a hospital and occurred in spring 2012 [6, 7]. Both proven cases in this hospital outbreak were fatal, and the other 9 (unproven) cases included 8 healthcare workers. While the clinical course of the index case has been described in some detail [5], and both renal and respiratory failure have been common, there have been no published reports of histopathologic findings, no autopsy reports, and minimal clinical information about the other 8 cases. And while it is likely that epidemiologic study is ongoing, there is currently only the most rudimentary information about exposures, animal contacts, or other epidemiologic aspects. The WHO is taking this virus very seriously

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and has published a case definition (including clusters of severe pneumonia without alternative explanation) for those who should undergo PCR testing in order to identify the HCoV-EMC that was identified in all the proven cases [8].

There is, interestingly, quite a lot of new information about the putative bat origin of HCoV-EMC. Initially, bat CoVs HKU4 and HKU5, both recovered from members of the Vespertilionidae family of bats in China, were considered the most likely ancestors of HCoV-EMC [5]. More recently, European investigators have found CoVs that are even more closely related to HCoV-EMC in *Pipistrellus* bats (members of the Vespertilionidae family) in the Netherlands (1 strain) and in eastern Romania and Ukraine (39 strains). The polymerase amino acid sequence of the most closely related bat virus (VM314) differs from that of HCoV-EMC only by 1.8% (as opposed to the sequence of CoV HKU5, which differs by 5.5%–5.9%) [9].

Besides extensive sequence data, there is almost no published information on the biology of HCoV-EMC or its pathogenicity in human or animal models. One welcome study is a report on receptor use in tissue culture: unlike SARS-CoV and HCoV-NL63, HCoV-EMC does not use angiotensin converting enzyme 2 as its receptor. However, it is in this information environment (many details on RNA sequence, few on the pathogenesis of infection, and almost none on epidemiology) that we need to interpret the article of Chan et al on the tissue culture tropism of HCoV-EMC.

The authors describe the “growth” of HCoV-EMC in a large number of cultured human cell lines and types, as well as in several animal cell lines. Growth in this study was measured by quantitative reverse transcription PCR of supernatant fluids (viral RNA, or “viral load”), as well as cytopathic effect and semiquantitative immunofluorescence of intracellular nucleoprotein stained with a polyclonal animal antiserum. It is unfortunate that infectious virus was never measured in

any of the cell lines tested and that evidence of virus replication depended either on production of viral RNA released into the supernatant fluid (“viral load”) and/or production of intracellular viral nucleoprotein detectable by immunofluorescence. Thus, virus “growth” as such was never shown. However, assuming that the authors’ assumptions that viral RNA and nucleic acid are adequate surrogates of new infectious virus, then the authors showed that the HCoV-EMC grew in several cell lines of lung origin—Calu3 cells (a line of cells derived from an adenocarcinoma arising from submucosal serous gland cells) [10] and human embryonic lung fibroblasts—as well as in cells originating from the human gastrointestinal tract, kidney, and liver and in a human histiocyte cell line. The virus also grew (using the same criteria) in monkey cells derived from kidney tissue (Vero and LLCMK2 cells), as well as in cells from pig kidney and civet lung.

What inferences can be made from such in vitro virus-cell tropisms regarding either the organ tropism of the virus in vivo or the pathogenesis of the viral infection? And what about similar inferences regarding species tropism from the species of origin of the various cell lines tested? For decades virologists have used this kind of inferential reasoning going both ways—in guessing what sorts of cells to test for growth of viruses in vitro, as well as in trying to work out the pathogenesis of infection—but these are dangerous waters. There are numerous examples even within the field of coronavirology in which the behavior of viruses in cells grown on plastic or glass does not appear to reflect their behavior in the intact organism. HCoV-229E was first isolated in secondary human embryo kidney cells, yet kidney pathology is not part of the illness caused by this virus [11]. Similarly, other strains of HCoV-229E were most readily isolated from patient samples by using a semicontinuous line of human embryonic intestine, HEI (or MA177), even though this virus does not cause diarrhea and is not isolated

from stool [12]. Cells in vitro can, by careful manipulation of their growth conditions, be induced to behave more or less like cells in vivo, but this also is a complex area, particularly when the cells are polarized, ciliated, or otherwise specialized in vivo, like the cells lining the respiratory tract.

Species tropism (as opposed to organ tropism) may be somewhat more specific, but even here there are complexities. Does growth of HCoV-EMC in pig kidney mean that the pig is a natural host for this virus? The civet? Perhaps, but, in fact, the most intriguing finding regarding species the tropism of HCoV-EMC was in a recently published study from European investigators showing that HCoV-EMC, unlike SARS-CoV, grew readily in cell lines obtained from several bat species [13]. In contrast, other HCoVs, including SARS-CoV, have shown very limited tropism for bat-derived cells. Thus, when added to the sequence data quoted above, the putative bat origin of this new CoV stands on somewhat firmer ground than that of SARS-CoV.

Similarly, Chan et al attempt to infer from the speed and extent of cytopathic effect in tissue cultures the virus’s virulence in vivo. This also is risky. Examine, for example, varicella-zoster virus (VZV) and herpes simplex virus (HSV), 2 closely related viruses that produce equally devastating systemic disease in newborns and immunodeficient humans. One (HSV) produces rapid (in hours), lytic and/or syncytial cytopathic effect in many different tissue cultures. The other (VZV) grows only in a few tissue culture types and even in these replicates very slowly, producing a cytopathic effect that is often difficult to discern.

Thus, although it is tempting to do so, it seems difficult to fill in many of our gaps in knowledge of the epidemiology and pathogenesis of severe HCoV-EMC-induced pneumonia with any sense of assurance through studies in tissue culture. Nonetheless, the work of Chan et al is useful in demonstrating the wide tissue tropism in vitro of HCoV-EMC.

Meanwhile, we await more information on the epidemiologic, clinical, and pathologic findings from the cases that have occurred so far and applaud the rapidity with which investigators have identified this threat and disseminated the available information.

Notes

Note added in proof. As of the current date (4 April, 2013), the World Health Organization has reported 17 cases of HCoV-EMC infection, with 11 deaths. All but 2 of these (both related to the case hospitalized in Birmingham, UK) have originated in the Middle East.

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