COMMENTARY

Moving beyond metagenomics to find the next pandemic virus

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Movements of viruses from animals to humans underlie outbreaks of diseases, such as Ebola hemorrhagic fever, influenza, and Middle East respiratory syndrome. The severe acute respiratory syndrome (SARS) virus pandemic of 2003 was caused by a novel coronavirus (CoV) that originated in Chinese horseshoe bats (1). Results of sequence analyses have shown that viruses related to SARS-CoV continue to circulate in bats, but their potential for infecting humans is not known. Gazing at viral sequences has its limits; experiments need to be done. In PNAS, Menachery et al. (2) develop a framework for deriving viruses from these genome sequences and examining their potential to cause the next SARS pandemic (Fig. 1).

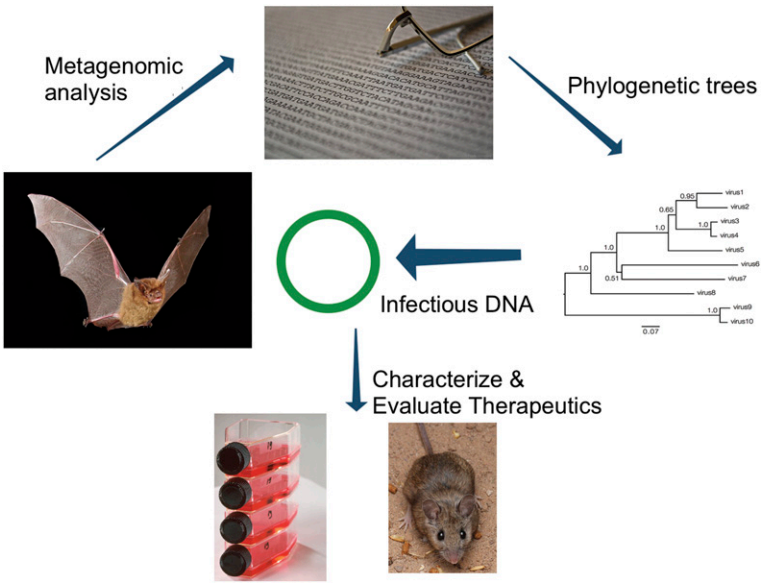

Fig. 1. Experimental platform for moving beyond metagenomics to identify viruses with pandemic potential. Samples from animals are subjected to deep, high-throughput sequencing to identify viral genomes. Sequence data are subjected to phylogenetic analyses to identify evolutionary relationships. Selected viral genomes are synthesized as DNAs and transfected into cells to recover virus. Viruses are evaluated for the ability to replicate in relevant human cell culture models and to cause disease in animal models. The latter can also be used to evaluate therapeutics (antivirals and monoclonal antibodies) and vaccines. Images courtesy of (clockwise, *Left* to *Right*) Flickr/Michael Pennay, Flickr/Pablo Gonzalez, Andrew Rambaut, Flickr/J. N. Stuart, and the National Cancer Institute.

Entry of SARS-CoV into human cells begins with binding of the viral spike glycoprotein to the cell surface receptor human angiotensin converting enzyme 2 (ACE2) (3). Most of the presumed ancestors of SARS-CoV found in bats are unable to bind this receptor (4). Several years ago in China, a bat CoV, WIV1-CoV, was found to bind to human ACE2 and replicate in human cells (1).

To determine if WIV1-CoV has the potential to infect humans, Menachery et al. (2) synthesized a DNA copy of the genome and introduced it into cells to recover infectious virus. The virus replicated as well as SARS-CoV in differentiated primary human airway epithelial cell cultures, the closest model to the human lung. These findings demonstrate that WIV1-CoV does not require adaptation for efficient replication in human cells.

An important question is whether WIV1-CoV causes disease in an animal model of infection. SARS-CoV does not cause disease in mice, but multiple passages of the virus in this host produced a mouse-adapted virus called SARS-CoV MA15. This virus induces rapid weight loss and lethality by 4 d after intranasal infection (5). When Menachery et al. (2) substituted the spike glycoprotein gene from SARS-CoV MA15 with the corresponding gene from WIV1-CoV, the resulting virus replicated poorly in mice, and did not cause weight loss or lethality. When wild-type viruses (e.g., not mouse-adapted) were inoculated into mice, neither SARS-CoV nor WIV1-CoV caused weight loss or lethality. However, the two viruses differed markedly in their ability to replicate in mice: SARS-CoV replicated to higher titers in the lung and brain compared with WIV1-CoV. These observations show that although the spike glycoprotein of WIV1-CoV can mediate entry of the virus into human cells, the virus does not cause disease in mice.

A very different outcome was observed when transgenic mice that produce the human ACE2 receptor were used by Menachery et al. (2). In contrast to wild-type mice, intranasal infection of these mice with SARS-CoV did result in weight loss and death. However, WIV1-CoV replicated to lower titers in ACE2 transgenic

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mice, and caused weight loss in fewer animals. Nevertheless, titers of WIV1-CoV in the lungs and brain were 100-fold higher than in wild-type mice, a likely consequence of the presence of human ACE2 receptor. These results underscore the limitations in using wild-type mice to study SARS-CoV pathogenesis.

The experimental findings suggest that WIV1-CoV is a potential threat to humans. If this virus emerged as a human pathogen, would we be prepared to prevent or treat infections? To answer this question, Menachery et al. (2) identified monoclonal antibodies against SARS-CoV that block infection of cells in culture by WIV1-CoV. To determine if these antibodies could prevent infection in an animal, human ACE2 transgenic mice were injected with antibody, and 1 d later infected intranasally with SARS-CoV or WIV1-CoV. One antibody blocked replication of both viruses in the lung, and protected mice from weight loss and lethality.

An important conclusion of Menachery et al. (2) is that a mixture of antibodies that block SARS-CoV infection might be used to protect humans if WIV1-CoV entered the population. Identification of a panel of antibodies that can block infection with ACE2-binding CoVs in bats should be an immediate research goal. Neutralizing antibodies are already used to treat human viral infections (e.g., rabies virus), and ZMapp, a mix of antibodies that block Ebolavirus infection, was used experimentally during the 2014–2015 outbreak in West Africa.

It would also be useful to have a vaccine ready in the event that WIV1-CoV or a similar virus enters the human population. A previously developed formalin and UV light-inactivated, whole virus preparation of SARS-CoV protected against infection in young mice. However, aged mice were not completely protected and showed evidence of increased immune pathology (6). When these immunized mice were challenged with WIV1-CoV, no weight loss or lethality was observed, but viral replication was not reduced compared with unimmunized animals, and was accompanied by eosinophilia. The antibodies induced by immunization with the inactivated SARS-CoV also failed to block infection by WIV1-CoV in cells. If WIV1-CoV or a similar virus were to spread in humans, vaccination with inactivated SARS-CoV would not protect against infection.

Previously, Menachery et al. used similar approaches to determine the pathogenic potential of another SARS-like bat virus called SHC014 (7). A recombinant virus was created in which the gene encoding the spike glycoprotein of mouse-adapted SARS-CoV virus was swapped with the gene from SHC014. The recombinant virus, called SHC014-MA15, replicated well in a human epithelial airway cell line and in primary human airway epithelial cell cultures. This virus was attenuated in mice. However, anti–SARS-CoV monoclonal antibodies did not protect from infection with SCH014-MA15, nor did immunization with inactivated SARS-CoV. SCH014 virus was recovered from an infectious DNA clone made from the genome sequence. This virus infected primary human airway epithelial cell cultures, but not as well as SARS-CoV. In mice, SCH014 did not cause weight loss and it replicated to lower titers than SARS-CoV.

The emergence of pandemic SARS-CoV in 2003, MERS-CoV in the Middle East, and influenza viruses and Ebolaviruses from animal sources emphasize the need to develop approaches for identifying viruses that could potentially initiate new outbreaks. The platform described by Menachery et al. (2) in PNAS, comprising metagenomics data, synthetic virology, transgenic mouse models, and monoclonal antibody therapy is an important advance in allowing assessment of the ability of SARS-CoV–like viruses to infect human cells and cause disease in mouse models. The results indicate that a bar SARS-like virus, WIV1-CoV, can infect human cells but is attenuated in mice. Additional changes in the WIV1-CoV genome are likely required to increase the pathogenesis of the virus for mice. The same experimental approaches used in the Menachery et al. paper could be used to examine the potential to infect humans of other animal viruses identified by metagenomics surveys.

It is essential to determine which genome changes could increase the pathogenicity of WIV1-CoV in mice. Adaptation of WIV1-CoV to mice would reveal sequences needed for virulence and for moving from a bat to another host. This information could not only be used to identify mutations of potential concern in bat viruses, but would also provide fundamental mechanistic information on how bat CoVs become more pathogenic. A variety of experimental approaches could be used to create such viruses, including animal passage and mutagenesis. However, it is likely that such experiments would not be permitted under the current moratorium on gain-of-function studies involving influenza virus and coronaviruses (https://www.whitehouse.gov/blog/2014/10/17/doing-diligence-assess-risks-and-benefits-life-sciences-gain-function-research).

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The current government pause on these gain-of-function experiments was brought about in part by several vocal critics who feel that the risks of this work outweigh potential benefits. On multiple occasions these individuals have indicated that some of the SARS-CoV work discussed in the Menachery et al. (2) article is of no merit. Such conclusions are inaccurate representations of the substantial advances provided by this work. As a consequence of these experiments with bat CoVs, we know that at least two of these circulating viruses can infect human airway cells, that vaccines do not prevent infection, and that monoclonal antibodies might be used to treat infection with at least one of these viruses should it enter the human population. These findings provide clear experimental paths for developing monoclonal antibodies and vaccines that could be used should another CoV begin to infect humans.

The critics of gain-of-function experiments frequently cite apocalyptic scenarios involving the release of altered viruses and subsequent catastrophic effects on humans (8). Such statements represent personal opinions that are simply meant to scare the public and push us toward unneeded regulation. Virologists have been manipulating viruses for years—this author was the first to produce, 35 y ago, an infectious DNA clone of an animal virus (9)—and no altered virus has gone on to cause an epidemic in humans. Although there have been recent lapses in high-containment biological facilities, none have resulted in harm, and work has gone on for years in many other facilities without incident (10). I understand that none of these arguments tell us what will happen in the future, but these are the data that we have to calculate risk, and it appears to be very low. As shown by Menacherry et al. (2) in PNAS, the benefits are considerable.

A major goal of life science research is to improve human health, and prohibiting experiments because they may have some risk is contrary to this goal. Being overly cautious is not without its

own risks, as we may not develop the advances needed to not only identify future pandemic viruses and develop methods to prevent and control disease, but to develop a basic understanding of pathogenesis that guides prevention. These are just some of the beneficial outcomes that we can predict. There are many examples of how science has progressed in areas that were never anticipated, the so-called serendipity of science. Examples abound, including the discovery of restriction enzymes that helped fuel the biotechnology revolution, and the development of the powerful CRISPR/Cas9 gene-editing technology from its obscure origins as a bacterial defense system.

Banning certain types of potentially risky experiments is short sighted and impedes the potential of science to improve human health. Rather than banning experiments, such as those described by Menachery et al. (2), measures should be put in place to allow their safe conduct. In this way science's full benefits for society can be realized, unfettered by artificial boundaries.

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