Response to the "Proximal Origins" of SARS-CoV-2

Abstract

This article is a response to "The Proximal Origin of SARS-CoV-2," published March 17, 2020 in *Nature Medicine*, which takes a limited view of the history of science and does not acknowledge the dual-use gain-of-function research practice of passing viruses either through hosts in vivo or in vitro using cell cultures. This practice of serial passage offers alternative explanations for both SARS-CoV-2's distinctive spike-protein region and the notable polybasic furin cleavage site within it. Additionally, we note that the existing serological research on exposure to bat coronaviruses does not indicate widespread infection of human populations, since only two-point-seven percent (2.7%) of villagers living about a kilometer from bat caves carried any evidence of past infections, and no one sampled the in city of Wuhan showed any past exposure at all.

Introduction

The article "The Proximal Origin of SARS-CoV-2", published March 17, 2020 in *Nature Medicine*, concludes based on detailed sequence analysis that the origin of the novel coronavirus causing the current pandemic is "not a laboratory construct nor a purposefully manipulated virus," but is more likely the result of natural selection and a zoonotic jump. The evidence presented includes two distinctive features possessed by the SARS-CoV-2 spike-protein (S-protein): the unique sequence in the receptor binding domain of S, a region known to be critical for SARS-CoV usage of human ACE2, the human cell surface receptor used by both SARS-CoV and SARS-CoV-2 for fusion with target cells and efficient cell entry. Also of note is the presence of a polybasic furin cleavage site in SARS-CoV-2 S-protein that is not found in SARS-CoV or other type B coronaviruses, but which is an important virulence feature observed to have been acquired by fusion proteins of avian influenza viruses and Newcastle Disease Virus grown under experimental conditions.

We challenge the principal conclusions of this paper, since the assigned likelihood of a natural origin for SARS-CoV-2 does not logically follow from the very evidence the authors introduce and cite. Additionally, without incorporating the history and implications of serial viral passage either through lab animals in vivo or through cell cultures invitro, it is impossible to accurately evaluate the possibility that this novel coronavirus is the result of a laboratory leak.

Discussion

"The Proximal Origin of SARS-CoV-2" leaves out the implications of the dual-use gain-of-function research tool of serial passage through animal hosts, a process likely applied to the strain of H1N1 Swine Flu, a variant of the pandemic influenza virus that leaked out of a Soviet

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lab in 1977 which is considered the product of "sequential passage in an animal reservoir,"[1] also the source of the mammal-transmissible H5N1 Bird Flu strain of influenza virus created about a decade ago, that was an airborne and highly virulent strain that "could change history if it was ever set free."[2]

The artificial manipulation of the H5N1 Bird Flu was contentious enough to cause the scientists working on it to "brace for a media storm,"[3] which occurred after researchers at two different institutions tweaked the H5N1 Bird Flu's genome in a few places and then passed it through a series of ferret hosts until it became both airborne and incredibly virulent,[4] and left behind a genome that appeared to be the result of natural, albeit accelerated, selection. That process of sequential passage through animal hosts leaves a genome that appears "natural" and not purposeful, since it does not leave a genomic smoking gun, and would appear to be the result of natural selection so long as its relationship to sister species is ignored. However, the artificial generations caused by forced serial passage through ferret hosts creates the appearance of evolutionary distance, which is exactly what is found with SARS-CoV-2 – so distant from any relatives that it has been given its own clade.[5]

If, as the authors propose, SARS-CoV-2 has a novel optimal solution for S-protein binding to ACE2, and if, as they suggest, this might have occurred by serial mutational events either in an intermediate host or after a zoonotic jump in humans – then logically it could also have occurred by the selection of a virus after serial passage through laboratory cultures or laboratory animals. The only origin for the SARS-CoV-2 S-protein RBD that the sequence data excludes is deliberate introduction of the SARS-CoV S-protein RBD sequence to create SARS-CoV-2. Otherwise, there are no structural or functional data to distinguish among natural and engineered possibilities at the present time. Regarding the second point, if other viruses have been observed to acquire polybasic cleavage sites by passage under experimental laboratory conditions, then obviously such a mechanism is possible for SARS-CoV-2. Although we congratulate the authors on the outstanding sequence analysis, we hope that the scientific community will continue to consider all possible origin mechanisms not yet excluded.

Notably, as the authors admit, there is another avenue for SARS-CoV-2's distinctive polybasic cleavage site to emerge other than through the mixing of unidentified viruses: "The acquisition of polybasic cleavage sites… has also been observed after repeated passage in cell culture or through animals." This is precisely the process understood to have led to the emergence of the H1N1 Swine Flu in the 1970s, as well as the aforementioned airborne and highly virulent gain-of-function enchanced H5N1 Bird Flu a decade ago, and remains a viable way for SARS-CoV-2 to have gained its polybasic cleavage site. And in very specific wording, other researchers have observed that this cleavage site may "provide a gain-of-function to the 2019-nCoV for efficient spreading in the human population compared to other lineage b betacoronaviruses."[6]

SARS-CoV-2 was clearly not built nucleotide-by-nucleotide as the perfect bespoke bio-weapon. But the "efficient solution" for ACE2 binding described by the authors as something that would not be intentionally engineered, is in fact exactly the result that could be selected for after serial passage through ferrets in lab, which was already done to the H5N1 Bird Flu to create an extraordinarily virulent and airborne strain. And studies examining SARS-CoV-2's infectivity in ferrets found that it spreads readily among them, and also appears airborne in that animal model,[7]

lending support to the idea that ferrets were used for serial passage. It should also be noted that several years prior to tinkering directly with bat coronavirus spike-proteins,[8] Dr. Ralph Baric supervised "synthetic reconstruction" of the SARS coronavirus that involved isolating a coronavirus from civets and then passing it through mammalian ACE2 receptor cells – serial passage through host cell lines instead of entire hosts, which imparted a strong affinity for ACE2,[9] and another novel strain of coronavirus that presumably was also airborne.

Additional support for possibility that serial passage through lab animals played a role in the creation of SARS-CoV-2 comes from an April 2020 pre-print, which found that it binds with ferret cells more tightly than any other species except the tree shrew, which only scored about 2% higher. Pangolins however, formed a much weaker bond than either, and were clustered way down on the list along with a handful of other much more unlikely intermediate animal hosts.[10] Further increasing the odds that serial passage was involved in the genesis of this novel coronavirus is the fact that tree shrews have also been used for serial viral passage, and were promoted in a 2018 paper out of China as a preferable animal host for laboratory serial passage since they're cheaper, smaller, easier to handle, and closer to humans evolutionarily and physiologically than ferrets. [11]

Further occluding the natural appearance of SARS-CoV-2, a recent study that examines the neutral sites that are assumed to best show heritage found that pangolins are "very unlikely" to have served as a host at all.[12] However, the authors note that "for a precursor virus to acquire both the polybasic cleavage site and mutations in the spike protein suitable for binding to human ACE2, an animal host would probably have to have a high population density." The ferret and tree shrew ACE2 receptor is roughly equivalent to the one found in humans, and they would certainly have a high population density if they were collected in a lab for serial passage gain-of-function research.

Addressing the possibility of long-standing circulation of bat-derived coronaviruses in humans, the authors cite a study published March 2018, that examined people who live in villages barely a kilometer away from bat caves. That study revealed that only two-point-seven percent (2.7%) of those villagers had antibodies indicating any past exposure to bat coronaviruses. That study also sampled people living in Wuhan and found no evidence whatsoever of exposure to "SARS-CoV-like coronaviruses."[13] So there is very little evidence of any exposure to these coronaviruses even in Chinese villagers living in close proximity to bat caves, and at the epicenter of the current outbreak – no exposure was found at all. These data do not support that SARS-CoV-2 was circulating in humans prior to the outbreak began in Wuhan in the winter of 2019.

Conclusion

It seems misguided and dangerous to ignore significant portions of the history of science, especially when that history is some of science's most troubled. It was the accidental leak of the H1N1 Swine Flu in the 1970s that first began the discussion about the moral and physical hazards involved with dual-use gain-of-function research. And the creation of the extraordinarily

virulent H5N1 Bird Flu, using the same technique of serial passage through an animal host in a lab, that helped lead to the NIH imposing a moratorium on dual-use gain-of-function research from 2014 until 2017, when it was relaxed explicitly to allow influenza as well as coronaviruses to be studied.[14]

Until a natural origin of SARS-CoV-2 is fully established, the prospect that it may have been the result of this research practice must be considered, especially since serial passage through ferret hosts explains every single element of its unusual genome and sudden emergence.

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