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Coronavirus SARS-CoV-2: filtering fact from fiction in the infodemic

Q&A with virologist Professor Urs Greber

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As the severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) continues to spread across the world, and the associated lung disease COVID-19 remains difficult to treat, information from media and private communication flows at high speed, often through unfiltered channels. Much of this information is speculative, as it derives from preliminary and inconclusive studies, and creates confusion as well as anxiety. This phenomenon was recently labelled as 'infodemic' by the World Health Organization.

We interviewed Dr. Urs Greber, Professor of Molecular Cell Biology and Principle Investigator in Virology at the Department of Molecular Life Sciences of the University of Zurich, Switzerland, to answer some of the most controversial questions about SARS-CoV-2 and set the facts straight.

Where did this virus come from?

Coronaviruses (CoVs) are endemic viruses in the human population. About 10–20% of the colds we get every year are due to coronaviruses, and they normally cause only minor problems.

However, SARS-CoV-2 is clearly more infectious and deadly to humans than the other endemic coronaviruses. SARS-CoV-2 efficiently replicates in the upper respiratory tract and can proceed into the lower respiratory tract where it exacerbates pre-existing lung conditions. It infects human cells when the S protein located on the surface of the coronavirus particle binds with high affinity to a protein exposed on the surface of the cells in the respiratory tract. Binding occurs through the receptor-binding domain (RBD) of the S protein. SARS-CoV-2, akin to several other coronaviruses, uses angiotensin-converting enzyme-2 (ACE-2) as an entry port into a lung cell [1].

A recent publication discussed the available evidence on the emergence of SARS-CoV-2. It suggested that SARS-CoV-2 emerged naturally, through a recombination of at least two viruses: a bat β-coronavirus (β-CoV) (genus *Rhinolophus*) and a pangolin β-CoV, neither of which normally infects humans [2]. The bat β-CoV is 96% similar to SARS-CoV-2, but it has a divergent receptor-binding domain in the S protein (only 60% similar to SARS-CoV-2) and binds poorly to human ACE-2. This makes it unlikely to enter human cells. The pangolin β-CoV is only 90% similar to SARS-CoV-2, but the RBD of its S protein has 99% similarity with SARS-CoV-2, and it has high affinity to ACE-2 [3]. In fact, its affinity to ACE-2 is higher than that of the SARS-CoV-1 RBD, as indicated in two recent studies [4,5].

A likely scenario for the origin of SARS-CoV-2 is that the bat and the pangolin β-CoVs have infected one and the same organism (we do not know which one exactly), and their genes recombined, resulting in the insertion of the pangolin RBD into the S protein of the bat β-CoV. The RBD from the pangolin virus has additional implications for the infectious nature of SARS-CoV-2. It harbours a furin enzyme cleavage site. Cleavage of the S protein at a furin cleavage site facilitates the entry of many viruses, including influenza viruses and CoVs into human cells. The importance of proteolytic cleavage at the furin cleavage site for the zoonotic transmission of viruses is in part based on experimental data with the MERS-like CoV from bats, which cannot efficiently enter human cells, unless small amounts of trypsin protease are added to the virion to mimic the furin cleavage of the S protein [6]. Interestingly, the ability of the S protein to be cleaved by the cellular furin protease is readily lost when CoVs are propagated in cell cultures, as shown with a feline CoV [7]. This implies that the acquisition of the furin cleavage site might be a gain of function for CoVs, but is lost when virus is amplified in cell cultures (Scheme 1).

Recombination typically requires the coexistence of at least two CoVs in a single infected cell, a situation which is favoured by viral persistence, that is the maintenance of viral genomes in infected cells over long periods of time. With CoVs, this has been illustrated in a study, where scientists in Wuhan (China) analysed the nucleotide sequences in faeces from bats and found a range of novel coronaviruses with variable sequences from SARS-CoV-1 indicative of massive rearrangements of CoV genomes in bats [8]. The emergence of

SARS-CoV by mutations, recombination and viral persistence has been discussed at length in the past [9].

On a broader scale, cross-species transmission events of coronaviruses are nothing new and account for several animal and human diseases in the past 40 years due to bovine, canine, feline and porcine coronaviruses, as well as human coronavirus OC43 and human coronavirus 229E [9].

Why is SARS-CoV-2 more infectious than most coronaviruses?

Viral transmission is measured with the transmission factor, R0. To have a better idea, when R0 < 1 the virus will disappear from the population over time, whereas R0> 2 means that the spread is exponential. One of the earliest hotspots of the virus was on the Diamond Princess cruise ship off the coast of Japan. A study performed on that ship showed that when social distancing was not implemented in the early phase of the epidemic, the transmission factor of SARS-CoV-2 was in the range of 15. This means that each infected person on the boat transferred the infection on average to 15 uninfected individuals. This is a very high transmission factor, similar to measles virus, and highlights the importance of social distancing in limiting the spread of the COVID-19 [10].

Mechanistically, we do not know all the factors that contribute to the high transmissibility of SARS-CoV-2. The furin cleavage site in the S protein likely makes the virus more transmissible. There are a number of possible ways that furin cleavage can cause increased transmissibility: the S protein could more easily detach from the cells enhancing viral shedding into aerosols; it could enhance replication or assembly of the virus; or it could increase their replication in the upper respiratory tract, which makes the virus easily exhaled (i.e. it goes in the aerosols with high efficiency).

SARS-CoV-2 appears to affect primarily elderly people. Why do children hardly develop severe symptoms?

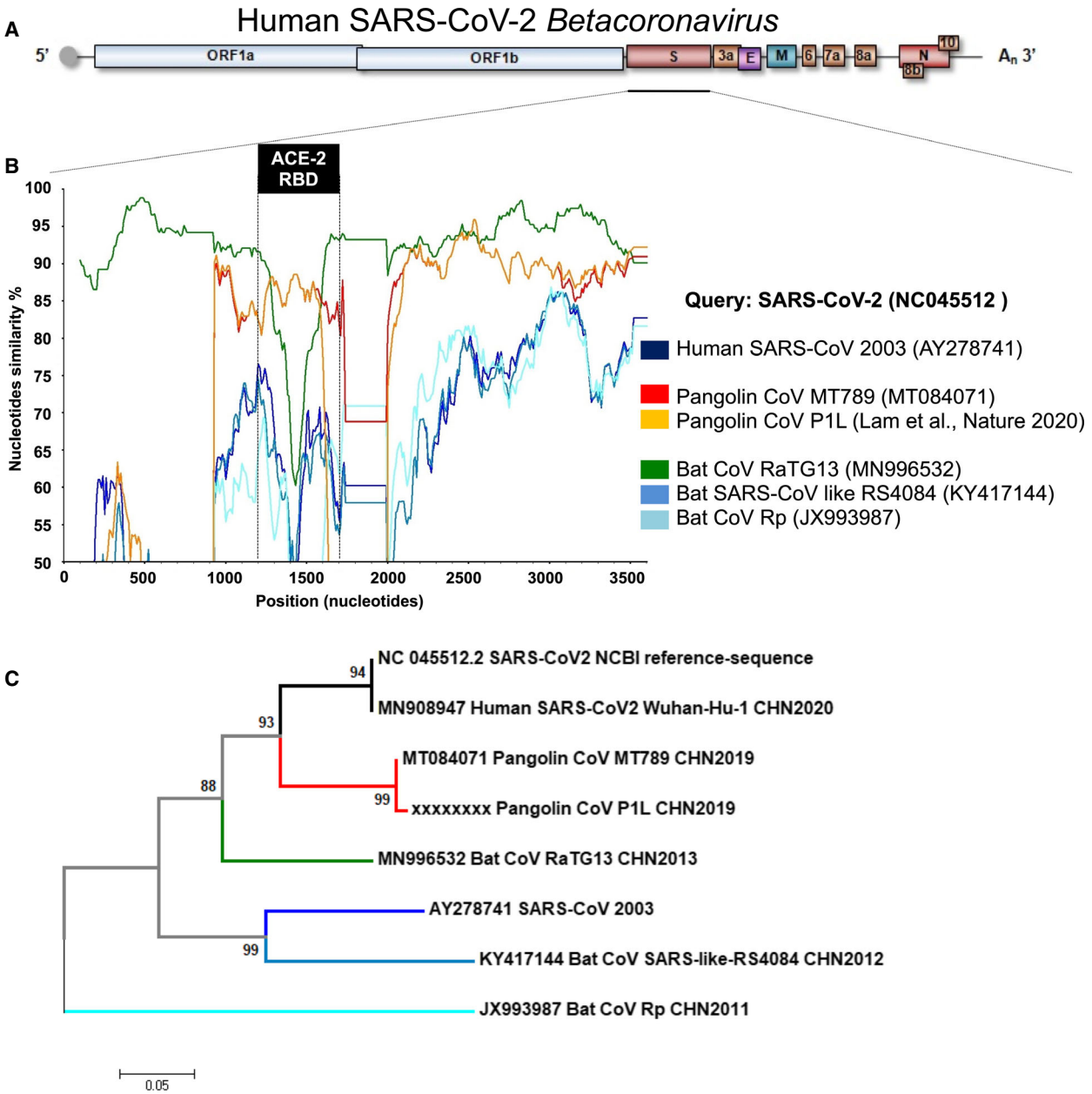
Statistics worldwide show that most of the deaths are among >65-year-old people. Children are basically just as likely as adults to get infected and to transmit the virus to other people. However, they develop milder symptoms, or none at all, and the mortality rate is practically zero. This has been observed for other coronavirus infections, but it is very different from influenza virus infections, where young children and the elderly are usually more severely affected.

No one really knows what protects children from coronaviruses. Possibly, T-cell responses in children lead to a better elimination of the virus (perhaps also via induction of different B cell responses or higher titre of antibodies) or a different type of inflammatory response. There might be differences in the innate phase of the immune response as well, such as production of different cytokines. There might also be a difference in the level of ACE-2 expressed on lung cells between children and adults, or even the shedding of soluble ACE-2, which might inactivate SARS-CoV-2. Understanding why children have a better immune response to COVID-19 may help develop effective therapeutic strategies for infected adults.

It appears that the severity of COVID-19 is different from one country to another and has increased over time. Is that true?

A recent study conducted in Beijing sequenced 103 SARS-CoV-2 genomes and classified them into two groups, L and S, based on 2 nucleotide differences between them [11]. They claim that there are two 'major types' (L and S) of SARS-CoV-2 and that these types have different transmission rates. These and other claims in this study are, however, highly controversial (for discussion see Ref. [12]). There is no convincing evidence that these two mutations are associated with the severity of the disease. Unless reverse genetics is used to study the questionable mutations, correlations between genetic changes and phenotypic changes in the course of an epidemic or pandemic are indirect, and strong claims are not justified.

Viruses replicate rapidly and accumulate mutations due to their error-prone viral polymerase, which allows them to adapt to changes in the immune system or different tissues in an organism. Yet, the coronaviruses encode several accessory proteins which reduce the error rate to about 1 in 0.5–2 million bases [13,14]. This means that only one out of 15–60 progeny viruses has a single point mutation in its genome. This is much lower than other RNA viruses and renders coronaviruses genetically rather stable, unless they recombine their genomes with a related CoV. In addition, the vast majority of the mutations have no phenotype, and they do not change viral infectivity. So, the good news is that SARS-CoV-2 has a low mutation rate (just one or two nucleotides a month, compared to four–eight nucleotides a month in influenza virus). Therefore, it is highly unlikely that the circulating virus has mutated to a more aggressive phenotype from November 2019 to March 2020.



Scheme 1. Genetic relationship of the SARS-CoV-2 spike (S) protein to bat and pangolin sequences as well as SARS-CoV-1. The scheme highlights the recombination breakpoint in the S protein gene of SARS-CoV-2. SARS-CoV-2 is 'very' similar to the Bat CoV RaTG13 S protein gene, except for the ACE-2 RBD, which is more sililar to the pangolin CoV (analyses by Simplot). (A) Schematic representation of the SARS-CoV-2 genome, which is a single-stranded (ss) RNA of positive polarity (+) around 30 kb, and codes for six open reading frames (ORFs) and accessory proteins. (B) Similarity plot analyses of different CoV S protein coding sequences. The SARS-CoV-2 NCBI reference nucleotide sequence (NC045512) was used as query. Sequences of the S protein from different CoVs are indicated in brackets with their respective animal origin, strain name and NCBI accession numbers. The ACE-2 RBD coding region is framed with dotted lines. The pairwise similarity between all sequences in a multiple sequence alignment (CLUSTALW; http://www.mbio.ncsu.edu/bioedit/bioedit.html) [20] was calculated with a 200-nt window moved along the sequence in 10 nt steps using the software SIMPLOT v3.5.1 [21]. (C) Neighbour joining analysis of the RBD coding region. Evolutionary distances were calculated by the Kimura two-parameter method, with 1000 bootstrap replications to estimate node consistencies. The length of the branch is proportional to the number of nucleotide divergences. Sequences are labelled with their respective accession number, animal origin, coronavirus strain and location/date of isolation. The sequence of pangolin CoV has been derived from [19]. The scheme was conceived and crafted by Dr. Romain Volle, Department of Molecular Life Sciences, University of Zurich, Switzerland.

Occasionally, mutations can be detrimental to viral infectivity. A study on SARS-CoV-1, the virus that caused the human pandemic in 2002–2003, showed that a deletion of 29 nucleotides in open reading frame eight occurred along the initial human-to-human transmission chain. Cell culture experiments showed that this mutation caused a severe loss of fitness [15]. As the 29-nucleotide deletion happened early on in the epidemics, it may have contributed to the disappearance of SARS-CoV-1 in 2003. Unfortunately, such a mutation may not help in resolving the current SARS-CoV-2 pandemic, as the wild-type strain is highly prevalent.

In conclusion, unfounded speculation about virulence increase of the circulating SARS-CoV-2 can be detrimental to the community, as it confuses people and accelerates irrational decision-making. There is absolutely no evidence as far as I understand that SARS-CoV-2 has become more aggressive in the course of the pandemic. Mortality rates may appear different due to different numbers of tests performed, different age groups tested, or different social behaviour between populations.

How stable can coronavirus be on particular surfaces?

Most studies on the stability of a virus on a surface are based on PCR, that is by detecting a small piece of intact viral genome. For example, biosafety officers who come to laboratories to take surface samples analyse them by PCR. A positive PCR result means that at least part of the virus is there, but this does not mean that it is infectious. Eike Steinman and colleagues wrote a review on the persistence of coronaviruses on different types of surface materials [16]. Infection studies showed that CoV can remain infectious on glass, plastic, iron and paper for hours to even a few days. The studies were performed with non-SARS coronaviruses, but the results are likely to be similar for SARS-CoV-2. In any case, you can inactivate the virus with soap, especially alkaline soap, or high concentrations of ethanol (> 60%).

What therapeutic approaches are being explored to defeat COVID-19?

A vaccine is definitely the best route as it can protect a large portion of the human population on an affordable basis. The low mutation rate of SARS-CoV-2 means that the chances of obtaining an effective and long-lasting vaccine are rather high. It will take 12–18 months to get the vaccine, however, unless someone finds a shortcut to make it faster. But shortcuts are dangerous since a vaccine failure may have devastating effects on society in terms of acceptance of vaccines in the future. We should only introduce a vaccine in humans after it has gone through animal trials, and we prove that it has antiviral efficacy.

Other therapeutic approaches involve the use of antivirals, such as remdesivir, which is a nucleoside analogue that inhibits the replication of the viral genome. It works very well in cell culture against SARS-CoV-1 and SARS-CoV-2 [17,18]. Other strategies explore the use of furin protease inhibitors. Researchers around the world are making strong efforts to prepare cocktails of monoclonal antibodies that can neutralise the virus. If this works, it can be scaled up and used for patients with acute infection, in order to reduce the viral load. However, this approach is expensive and may be difficult to distribute because it involves biologicals which are subject to denaturation if stored improperly, or not kept in a cold chain. In addition, it is unclear how long-lasting the effects of antivirals can be, because drug-resistant viruses will inevitably emerge. Nevertheless, direct antivirals will be useful, as they buy time while patients develop immunity.

Alternative approaches involve developing inhibitors that target physiological functions that are not viral, but cell-based, and are important for viral infectivity. For example, if we inhibit ACE-2, we can slow down viral entry. This of course will also inhibit the receptor's physiological function, but researchers believe the body could tolerate the inhibition. Yet, viruses have been shown to be able to switch cell receptors in some cases. Receptor-targeting approaches might not be effective in the long run. Finally, doctors are using an IL6 receptor blocker, developed by Roche for treating rheumatoid arthritis. This drug basically inhibits the inflammatory response, easing out the symptoms in people who are heavily infected with lower respiratory disease. Whether an overshooting inflammatory response is a universal feature of lower respiratory tract SARS-CoV-2 infection remains to be seen.

In summary, the COVID-19 outbreak is an indication of how difficult it is to handle emerging and reemerging infectious viruses. It teaches us the importance of accurate communication, constant surveillance, rapid diagnosis and fundamental research on any aspect of virology. This will help us to better counteract the emergence of new pathogens in the future.

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