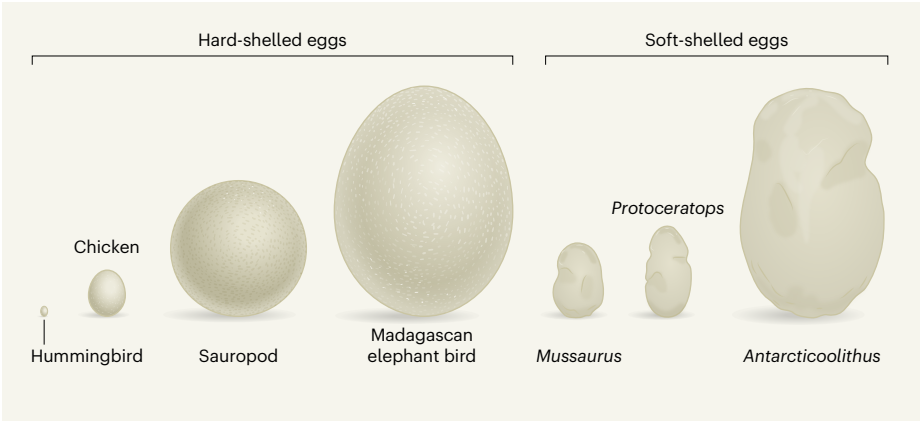

Figure 1 | Egg evolution. Hard-shelled eggs vary in size, from small eggs, such as that of a hummingbird or chicken, to the huge egg that belongs to the extinct Madagascan elephant bird, *Aepyornis maximus*. A few dinosaur groups, including sauropods, laid hard-shelled eggs. Norell *et al.*[1] report the discovery that two types of dinosaur laid soft-shelled eggs. The authors analysed *Mussaurus* eggs that are between 227 million and 209 million years old, and *Protoceratops* eggs of between 84 million and 72 million years old. This finding challenges the generally accepted view that dinosaur eggs were always hard-shelled, in turn suggesting that the earliest eggs laid by dinosaurs were soft-shelled. Legendre *et al.*[2] report the discovery of a huge originally soft-shelled egg in Antarctica, a specimen they call *Antarcticoolithus*, that is about 68 million years old. Legendre and colleagues hypothesize that this might have been laid by a marine reptile. However, Norell and colleagues' discovery raises the possibility that *Antarcticoolithus* was instead laid by a dinosaur.

specimen *Antarcticoolithus*, after the Antarctic continent and the ancient Greek words for egg and stone. *Antarcticoolithus* is among the largest eggs ever recorded (Fig. 1), being rivalled in volume only by those of some non-avian dinosaurs and the extinct Madagascan elephant bird, *Aepyornis maximus*. Notably however, these other egg types are characterized by thick calcareous shells, whereas *Antarcticoolithus* has a thin and presumably originally soft covering.

Although cautiously pointing out that no embryonic remains were found in the fossil egg, Legendre *et al.* hypothesize that it might have been laid by a giant marine reptile, and perhaps most feasibly a mosasaur, on the basis of structural similarities to the leathery eggs of lepidosaurs — the group that includes mosasaurs, living lizards, snakes, amphisbaenians (burrowing worm lizards) and the lizard-like tuatara, *Sphenodon punctatus*. Furthermore, because mosasaurs had streamlined bodies and thus were unable to move on land[8], Legendre and colleagues propose that egg laying must have taken place under some depth of water. Nevertheless, although modern viviparous lizards certainly give birth to fully developed young that are surrounded by thin coverings (mainly extraembryonic membranes)[9], the few known fossils of pregnant mosasauroids (the group containing mosasaurs and their ancestors) have not been found associated with eggshell debris[10]. Crucially, mosasaurs were also air breathers; therefore, laying a soft-shelled egg under water would have entailed a considerable risk of drowning for the emerging newborn.

Identifying the elusive producer of the *Antarcticoolithus* egg becomes even more intriguing given the findings of Norell *et al.*, which could implicate some form of dinosaur as the proud parent. Indeed, the total estimated weight of *Antarcticoolithus* clearly approaches those of the largest non-avian dinosaur and bird eggs, and both these groups have a history of fossil occurrences in Antarctica[11]. Dinosaur parentage thus at least seems plausible for *Antarcticoolithus*, which might have been laid on land and then washed out to sea as a discarded eggshell. This could have remained buoyant for some time because of trapped air, before finally sinking to the sea floor, where it was buried in sediment and eventually fossilized. Let us hope that future discoveries of similarly spectacular fossil eggs with intact embryos will solve this thought-provoking enigma.

Johan Lindgren is in the Department of Geology, Lund University, 223 62 Lund, Sweden. **Benjamin P. Kear** is at the Museum of Evolution, Uppsala University, 752 36 Uppsala, Sweden.
e-mails: johan.lindgren@geol.lu.se; benjamin.kear@em.uu.se

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Coronavirus

A race to determine what drives COVID-19 severity

Marios Koutsakos & Katherine Kedzierska

Efforts are ongoing to find which human or viral factors underpin whether a person with COVID-19 will develop severe symptoms. Clinical evidence linked to two viral lineages now provides key insights into this enigma. **See p.437**

The coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019, and certain aspects of the disease it causes — COVID-19 — continue to baffle clinicians and researchers. It is estimated that SARS-CoV-2 has already infected more than 9 million people and claimed more than 450,000 lives worldwide, and this pandemic has paralysed economies globally. On page 437, Zhang *et al.*[1] present data on the evolution of two major lineages of SARS-CoV-2, together with information regarding human-host determinants of disease severity from their analysis of 326 people in Shanghai, China, who were infected with SARS-CoV-2.

SARS-CoV-2, which caught the world by surprise, was initially thought to have 'jumped' to humans from an animal host at the Huanan Seafood Wholesale Market in Wuhan, China. When the first cases of a previously unknown disease, initially described as 'a severe

pneumonia with unknown aetiology', were identified in Wuhan at the end of December 2019, the majority of cases could be traced back to this market. The implication was that the new coronavirus had crossed the species barrier at the market from an infected live animal on sale. The Malayan pangolin, a scaly anteater previously living in relative obscurity, suddenly faced allegations that it was the culprit, although whether this protected creature was on sale in the market at that time is uncertain (see *Nature* http://doi.org/ggpxhb; 2020). However, some cases of the disease in early December 2019 in Wuhan had no obvious links to the market[2].

Zhang *et al*. analysed 94 complete genome sequences of SARS-CoV-2 in samples obtained from people living in Shanghai who had visited a health-care clinic in January or February 2020, and compared these data with 221 other sequences of the virus. The authors' results reinforce previous observations[3] of two major phylogenetic lineages (clades) of SARS-CoV-2 during the early phase of the outbreak in China. They are distinguished by two distinctive nucleotide differences, suggesting multiple origins for the human infections transmitted to people in Shanghai (which is about 800 kilometres by road from Wuhan).

The two lineages are termed clades I and II (Fig. 1). They presumably evolved independently from a common ancestor, but their ancestry in terms of how they relate to each other is unclear, because they differ at only two genomic sites. One difference involves a particular nucleotide in the sequence that encodes amino-acid residue number 84 in the viral protein ORF8. If the nucleotide has a thymine base (clade I), the sequence encodes the amino acid leucine; if it has a cytosine base (clade II), the sequence encodes a serine. The other difference is at a nucleotide in the gene *ORF1ab*, which contains either cytosine (clade I) or thymine (clade II); both the resulting nucleotide sequences encode serine.

Combining viral genomics with epidemiological evidence of how people might have picked up the infection, Zhang *et al*. show that the viral genomes from six people with established links to the Wuhan market cluster in clade I on the SARS-CoV-2 family tree, whereas the viral genomes of three cases without known links to the market cluster in clade II. These data support the idea that the market might not have been the origin of the pandemic. Instead, they suggest that clades I and II originated from a common viral ancestor and spread independently at the same time: clade I through the market and clade II outside it. Therefore, the animal-to-human transfer might have occurred elsewhere, seeding transmission chains that found their way to the market — where the high density of stalls and susceptible humans facilitated uncontainable spread in, and subsequently beyond, the site.

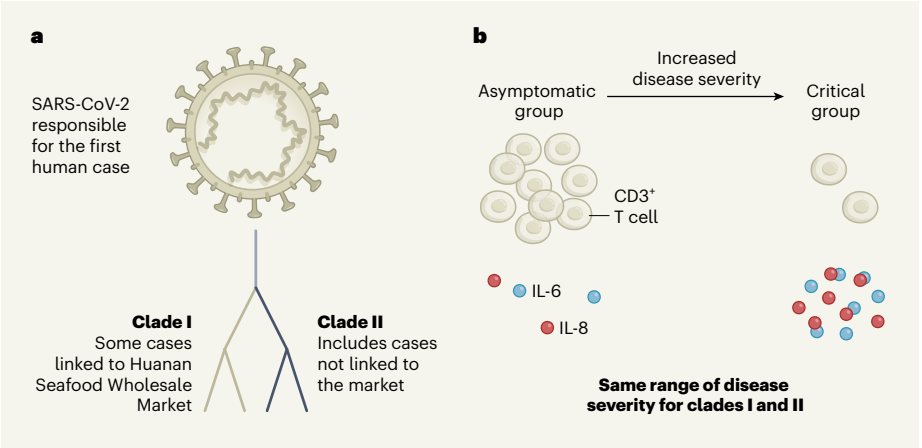

Figure 1 | Assessing the relationship between coronavirus lineages and COVID-19 severity. Zhang *et al*.[1] studied people from Shanghai, China, who were infected with the coronavirus SARS-CoV-2 in early 2020. **a**, Consistent with previous research[3], the coronavirus genome sequences Zhang *et al*. identified belonged to two lineages, termed clade I and clade II. These differ at two nucleotides and probably evolved independently from a common ancestor. Clade I was associated with some cases linked to Huanan Seafood Wholesale Market in Wuhan, China, originally thought to have been the source of the outbreak, whereas the authors found clade II infections that did not have links to the market. Both lineages might have spread independently at the same time. **b**, Zhang and colleagues categorized the individuals into four groups, depending on their disease severity, which ranged from those unaffected by symptoms (the asymptomatic group) to the critical group (those requiring artificial ventilation to breathe). Both clades had the same ability to cause the different disease groupings. An increase in disease severity was accompanied by a depletion of immune cells called $CD3^+$ T cells and an increase in the pro-inflammatory cytokine proteins IL-6 and IL-8. High cytokine levels can cause an intense immune response known as a cytokine storm.

The circulation of different 'types' of SARS-CoV-2 has been a contentious topic, stemming from the observation of distinct phylogenetic lineages. However, such genetic divergence among viruses, especially in the context of 'immunologically naive' human hosts (those who have never encountered the virus before) is expected. This can be explained by the 'founder effect', which is common during viral outbreaks — if a limited number of viral variants randomly enter a new geographical region where there is a susceptible population, their subsequent spread there facilitates the dominance of those variants at that location.

However, the difference in prevalence of those variants in that particular population, compared with infected populations in other regions, does not necessarily equate to improved fitness of those variants in terms of viral replication and transmission[4]. Consistent with this idea, Zhang *et al*. find no evidence of any association between either of the two clades, or between any mutations in subclades, and the clinical parameters they assessed to categorize COVID-19 disease severity. Although this finding is not surprising, given that the two clades differ by only two nucleotides out of the approximately 30,000 nucleotides in the SARS-CoV-2 genome, it highlights the fact that distinct phylogenetic lineages do not necessarily indicate distinct viral strains with different disease outcomes.

Having found no difference in clinical outcomes between infections with the two SARS-CoV-2 lineages, Zhang *et al*. analysed various parameters of immune-system function in the human hosts to identity factors that contribute to disease severity.

The authors focused on four disease categories with well described definitions of clinical outcomes. The least-affected individuals were asymptomatic and had no fever, no breathing problems and no signs of lung damage on X-ray scans. Mild cases were those in people who had fever and signs of inflammation on X-rays of their lungs, indicating pneumonia. People with severe disease had difficulty breathing and had hallmarks of lung damage described as 'ground-glass opacities' on X-rays. Critically ill patients had acute respiratory distress syndrome, and required mechanical ventilation to assist breathing. In agreement with previous research[5], Zhang and colleagues found that being older, the presence of other pre-existing medical conditions (termed comorbidities), and male gender were the leading factors associated with a higher probability of more-severe disease.

From the analysis of blood samples, the authors provide evidence of changes that characterized the severe and critical cases of COVID-19. One characteristic of these cases was lymphocytopenia — an abnormally low number of lymphocytes (a type of white blood cell involved in immune responses) in the blood. Zhang *et al*. attributed this lymphocytopenia to the depletion of a particular type of lymphocyte called a $CD3^+$ T cell, most probably reflecting movement of these T cells from the blood to sites of infection in tissues.

Another characteristic of the severe and critical cases was abnormally high levels of the cytokines IL-6 and IL-8, which are small proteins that promote inflammation. High levels of pro-inflammatory cytokines drive an intense immune response that is commonly referred to as a cytokine storm. Immune-system cells called macrophages, which are present in the lung, can make IL-6 and IL-8, and are often the initial cellular mediators of a cytokine storm in other respiratory infections. However, the precise cell populations contributing to the prolonged cytokine storm that occurs in some cases of COVID-19 remain to be defined.

The inverse correlation between high levels of IL-6 or IL-8 and low lymphocyte numbers hints at underlying mechanisms that might link these characteristics of severe disease. The possibility that high cytokine levels cause lymphocytopenia is consistent with the observation that people with COVID-19 who were treated with the drug tocilizumab, which blocks IL-6-mediated signalling, had their bloodstream levels of lymphocytes restored to nearer normal[6]. However, further experimental and mechanistic studies are needed to establish whether a causal connection underlies the correlation between these cytokine levels and lymphocytopenia. Of note is the discordant time frame of changes in these two parameters — T-cell depletion is evident from the first week of overt disease, whereas a cytokine storm arises later, when COVID-19 has become severe.

Moreover, neither lymphocytopenia nor a cytokine storm are exclusive to COVID-19. Both are hallmarks of many types of severe respiratory infection, including human infection by avian influenza viruses, and severe acute respiratory syndrome (SARS), a disease caused by a coronavirus related to SARS-CoV-2. To delineate the immunological signatures that are specific to COVID-19, more-detailed cellular and molecular analyses will be needed.

"The authors' work raises key questions that will need to be answered if we are to limit this pandemic."

Tracing the evolution of SARS-CoV-2 is fundamental for informing the public-health policies needed to limit disease spread. Dissecting the underlying causes and mechanisms of perturbed immune defences, such as the depletion of $CD3^+$ T cells and the heightened pro-inflammatory response, as well as determining the crucial clinical and molecular hallmarks of COVID-19, are of paramount importance for the design of treatment strategies and effective vaccines. Zhang *et al.* lay some essential groundwork that should aid in these Herculean tasks, and their work raises key questions that will need to be answered if we are to limit this pandemic and try to prevent a future one.

Marios Koutsakos and **Katherine Kedzierska** are in the Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Victoria 3010, Australia.
e-mail: kkedz@unimelb.edu.au

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