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PRIORITY PAPER

Asymptomatic SARS-CoV-2 infection: is it all about being refractile to innate immune sensing of viral spare-parts?—Clues from exotic animal reservoirs

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One sentence summary: The authors analyze factors regarding asymptomatic COVID-19 and discuss the importance of identifying key clues to the development of therapeutic versatility against SARS-CoV-2.

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ABSTRACT

A vast proportion of coronavirus disease 2019 (COVID-19) individuals remain asymptomatic and can shed severe acute respiratory syndrome (SARS-CoV) type 2 virus to transmit the infection, which also explains the exponential increase in the number of COVID-19 cases globally. Furthermore, the rate of recovery from clinical COVID-19 in certain pockets of the globe is surprisingly high. Based on published reports and available literature, here, we speculated a few immunovirological mechanisms as to why a vast majority of individuals remain asymptomatic similar to exotic animal (bats and pangolins) reservoirs that remain refractile to disease development despite carrying a huge load of diverse insidious viral species, and whether such evolutionary advantage would unveil therapeutic strategies against COVID-19 infection in humans. Understanding the unique mechanisms that exotic animal species employ to achieve viral control, as well as inflammatory regulation, appears to hold key clues to the development of therapeutic versatility against COVID-19.

Keywords: asymptomatic; COVID-19; evolution; origin; monocytes; SARS-CoV-2

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BACKGROUND

In December 2019, China reported an outbreak of acute respiratory distress syndrome (ARDS) due to an unknown etiology, which was epidemiologically linked to a wet market located in Wuhan of Hubei Province (Bar-On *et al.* 2020; Zhu *et al.* 2020). The causative agent was initially named as a Chinese novel coronavirus (2019-nCoV), and later named as SARS-CoV-2, which is responsible for the ongoing global coronavirus disease-2019 (COVID-19) epidemic (Zheng 2020). Until 15th December 2020, ∼72.7 million people were confirmed infected with SARS-CoV-2 with over ∼1.62 million confirmed deaths and recovery of 47.4 million individuals (Our World in Data Report—India 2020). In India, active infection has been reported in ∼9.88 million individuals and ∼0.143 million deaths, with only 99 deaths per million population (WHO Coronavirus Dashboard 2020, World Health Organization (WHO)) and is steadily increasing even at this time of editing the proof pages. It has also become apparent that the number of individuals recovering from COVID-19 is high, with India reporting a whopping total of ∼9.39 million individuals (∼95%) having successfully overcome the disease. Interestingly, there are dramatically high rates of asymptomatic human infections, and the scientific mechanisms underlying such conditions largely remain unexplored (Day 2020; He *et al.* 2020; Mizumoto *et al.* 2020; Rivet *et al.* 2020).

The incubation period (or pre-symptomatic period) for COVID-19 is reported to be between five and six days, although this can be up to 14 days or even higher. According to WHO definition, asymptomatic COVID-19 case refers to an individual who displays signs and symptoms compatible with SARS-CoV-2 infection, and symptomatic transmission refers to dissemination of the virus from a person who suffers symptoms. On the other hand, an asymptomatic laboratory-confirmed case refers to a person infected with SARS-CoV-2 who does not develop symptoms. Asymptomatic transmission refers to viral transmission from an asymptomatic individual (WHO COVID-19 Situation Report–73 2020). Interestingly, it has become apparent that pre-symptomatic individuals with SARS-CoV-2 infection could transmit the virus via infectious droplets or contact with fomite surfaces before development of symptoms, prior to testing positive for COVID-19 (Wei *et al.* 2020).

Observational investigations suggest that viral shedding is highest in the upper respiratory tract during the first 3 days of onset of symptoms (Lauer *et al.* 2020; Wang *et al.* 2020). Preliminary data suggested that infected individuals remain contagious around this time of symptom onset. Although the proportion of asymptomatic infections still need a thorough global population screening, some studies have estimated that up to 80% of individuals, diagnosed with the SARS-CoV-2 remain asymptomatic (Day 2020). Notably, these asymptomatic cases may even be higher considering that some of the cases might likely be missed by the recommended qRT-PCR testing (Long *et al.* 2020). In this study, 37 asymptomatic individuals in the Wanzhou District of China were investigated wherein the authors found that asymptomatic individuals had an extended median duration time of 19 days of viral shedding compared to the symptomatic individuals (Long *et al.* 2020). This emphasizes the seriousness of the role of asymptomatic individuals in the exponential increase in the number of COVID-19 cases globally (He *et al.* 2020; Mizumoto *et al.* 2020; Tindale *et al.* 2020). Furthermore, the virus-specific IgG and neutralizing antibody levels were significantly lower in the asymptomatic individuals as compared to symptomatic individuals during the acute phase of SARS-CoV-2 infection. The reduction in IgG and neutralizing antibody levels in the early convalescent phase of COVID-19 infection likely appears to have implications on immunity strategy and serological surveys.

Exotic animal species: The pandora's box of insidious viruses?

While the origin of SARS-CoV-2 largely remains elusive, available evidence points to the likely involvement of two exotic mammalian species: Malayan pangolins and bats (Du *et al.* 2008; Andersen *et al.* 2020; Fischer, Tschachler and Eckhart 2020b; Wu *et al.* 2020; Zhang, Wu and Zhang 2020; Zhou *et al.* 2020). It is widely believed that both the animal species could serve as the reservoirs of the β-coronavirus (β-CoV) from where the virus would have 'jumped' into the human compartment, and after adapting to the human system might have led to the COVID-19 outbreak to exponentially expand the ongoing pandemic manifold (Fischer, Tschachler and Eckhart 2020a; Zhang, Wu and Zhang 2020; Fig. 1). Whole genome sequencing suggests that pangolin-CoV and BatCoV RaTG13 are 91.02% and 90.55% identical to SARS-CoV-2, respectively (Zhang, Wu and Zhang 2020). It is presumed that the BatCoV upon entry into the pangolin system underwent homologous recombination (to become pangolin-CoV) before evolving into a human transmissible variant CoV in the scaly mammals (Ji *et al.* 2020). It is a classical fact that bats have been known to harbor some of the deadliest viruses on earth, for instance, SARS-CoV, MERS-CoV, Ebola, Marburg (filoviruses) etc (Zhang *et al.* 2013; Antonioli *et al.* 2020). Besides, bats are also predators of several mosquito species that transmit human infections. But how do these exotic animals remain 'tolerant' or asymptomatic despite the presence of insidious viruses, especially CoVs in them? Why do their cytotoxic cells (for instance, natural killer (NK) cells and CD8+ T cells) that are classically anti-viral remain mute spectators?

One mechanism postulated that bats have evolved to bypass exaggerated inflammation by limiting the assembly of the NLR family pyrin domain containing three (NLRP3) in monocytes (Papenfuss *et al.* 2012, Ahn *et al.* 2019). It also appears that bats enjoy the privilege of owning several key evolutionary adaptations (Shaw *et al.* 2012) having occurred in them over the last several thousand years whereby they seem to have lost the ability to effectively sense cytoplasmic DNA via loss of PYHIN genes and a regulatory site mutation in STING (Fischer, Tschachler and Eckhart 2020a), which remain otherwise in human (Fig. 2), which remain the underlying mechanism behind decreased levels of TNF-α and a plethora of other pro-inflammatory cytokines, especially IL-1β, IL-6, CXCL-8 and IL-18. It has also become clear that bats secrete excessively high levels of the anti-inflammatory cytokine IL-10 that has been known to 'silence' inflammation in bats (Kacprzyk *et al.* 2017). Evidence also suggests that bats being active animals of flight display enhanced autophagy that classically augments protection attributes against viruses (especially against bat lyssaviruses) in the mammals (Laing *et al.* 2019). Another mechanism points to the lack of functional killer cell Ig-like (KIR), and killer cell lectin-like (KLR) receptor loci used by classical NK cells (Wu *et al.* 2020). If so, do bat NK cells use alternate receptors to recognize MHC class I for activation and inhibition? If bats do possess granzyme-mediated cytolysins (Masselli *et al.* 2020) and antibody-dependent cellular cytotoxicity (ADCC; Mizumoto *et al.* 2020), why do the animals remain quiescent towards insidious viruses?

Interesting recent research suggests that pangolins have developed an evolutionary advantage as they appear to have lost an interferon-induced with helicase C domain 1 (IFIH1),

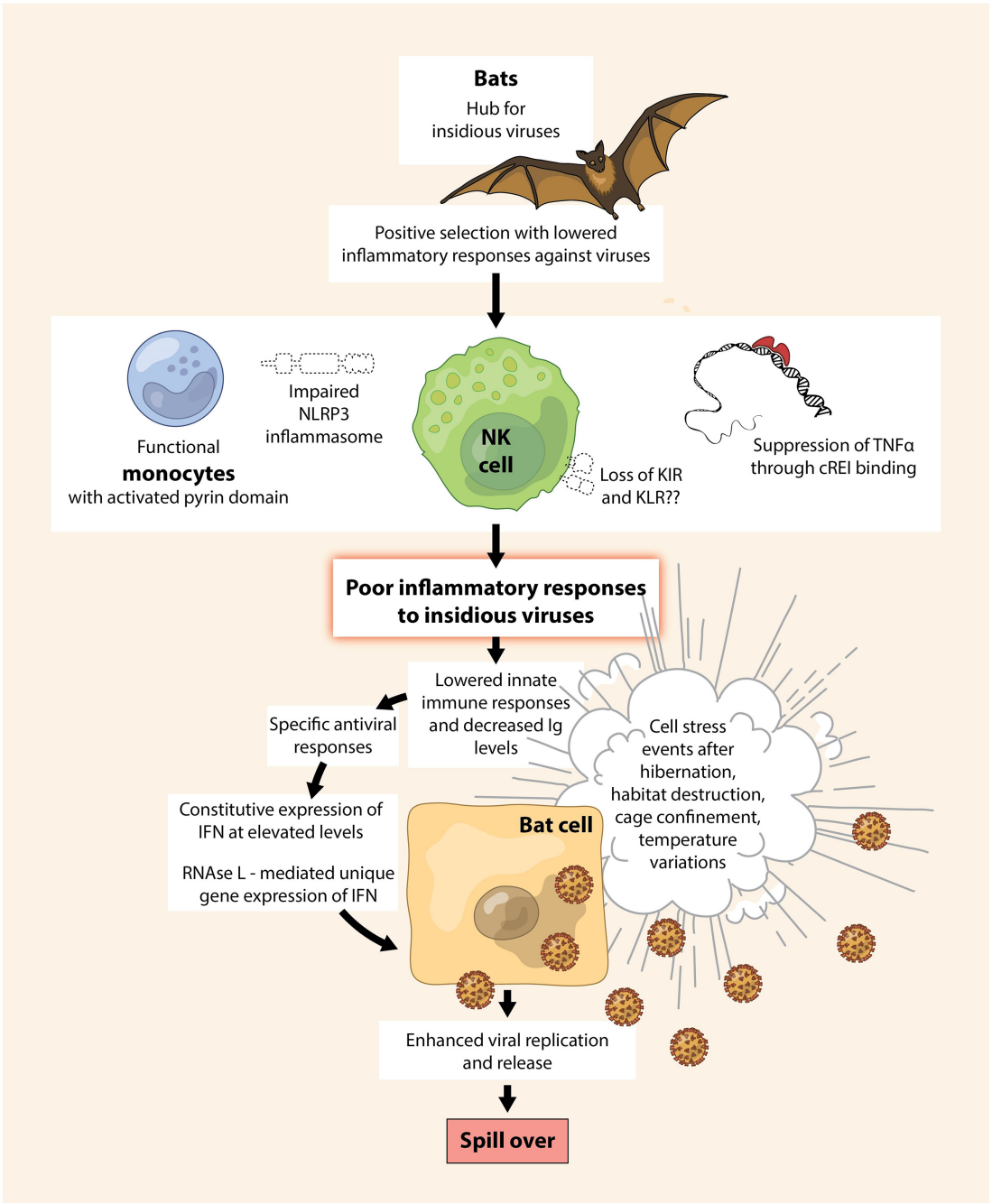

Figure 1. Proposed mechanism of existence of β-coronaviruses (β-CoV) in exotic mammalian animal reservoirs. Exotic bats serve as primary reservoirs of β-coronaviruses (β-CoV) from where the later appears to have 'landed' into pangolins (not shown) where the virus seems to have undergone a homologous recombination of nucleotide base pairs with insidious pangolin-CoV before getting into the human system (upon consumption or inhalation of virus present in body fluids), potentially initiating an outbreak. It also appears that bats bypass exaggerated inflammation by limiting the assembly of the NLR family pyrin domain containing three (NLRP3) in monocytes, and besides the paucity of functional killer cell Ig-like (KIR), and killer cell lectin-like (KLR) receptor loci expressed classically by NK cells.

also known as IFIH1/MDA5, and another protein, the Z-DNA-binding protein (ZBP1) that detects both Z-DNA and Z-RNA, which bypasses inflammation-induced damage of tissues during the presence of insidious viruses (Du *et al.* 2008). Interestingly, it has also been demonstrated that TLR5, an innate sensor of bacterial flagellin (Sharma *et al.* 2020; Qiu, 2020) and interferon-ε expressed in the epithelia of certain mammalian species, have been reported to have disappeared in pangolins, possibly during the course of evolution (Choo *et al.* 2016; Brook *et al.* 2020).

Asymptomatic COVID-19 and possible mechanisms

Emerging findings suggest that vast proportions of individuals with COVID-19 remain asymptomatic and can shed viruses to transmit infection (Day 2020; He *et al.* 2020; Masselli *et al.* 2020; Rivett *et al.* 2020) likely accounting for the exponential increase in the number of COVID-19 cases globally (Guan *et al.* 2020). Of the several pathogenesis mechanisms attributed to the onset of COVID-19, cytokine storm syndrome or hypercytokinema has taken center-stage owing to its

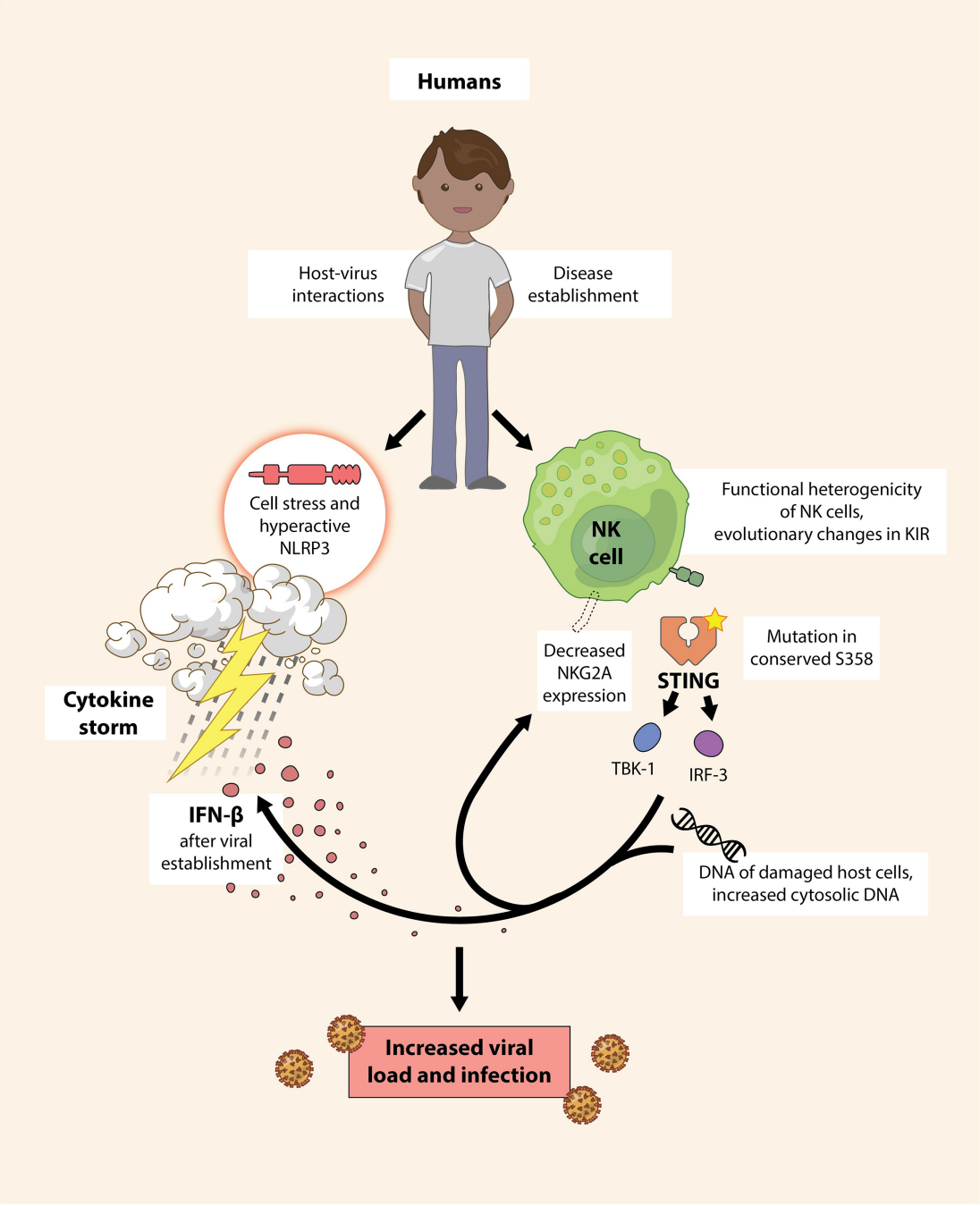

Figure 2. Mechanistic basis of proposed COVID-19 immunopathogenesis in humans. Notwithstanding the proportion of asymptomatic COVID-19 disease warrants an extensive global population screening, some studies have estimated that ~80% of individuals, diagnosed with the SARS-CoV-2 remain sub-clinical. Despite being an RNA virus, SARS-CoV-2 can activate the STING pathway (Fischer, Tschachler and Eckhart 2020a) to induce cytokine storm syndrome, also fueled by NLRP3 inflammasome activation and production of IL-1β, and IL-18, besides TNF-α, IFN-γ and IL-6 to deteriorate disease severity. A Chinese study has shown that asymptomatic individuals displayed an extended median duration of 19 days of viral shedding compared to symptomatic patients portraying the role of asymptomatic/subclinical disease status with the exponential upsurge in global COVID-19 cases (Long *et al.* 2020).

association with the onset of fatal pneumonitis and clinical deterioration (Giamarellos-Bourboulis *et al.* 2020; Girija, Shankar and Larsson 2020). Similar to monocytes that are believed to be responsible for the abrupt onset of cytokine storm syndrome, it has also been reported that the NK cell population become exhausted (Zheng *et al.* 2020). Lack of functional NK cells may likely allow viruses to proliferate intracellularly to trigger the NLRP3 activation. Further, evidence available thus far is also suggestive of NK cell cytopenia in patients with severe SARS-CoV-2 infection (Diao *et al.* 2020; Zheng *et al.* 2020). Also, as stated earlier, the function of NK cells has been correlated with the expression of NKG2A (CD159a), a well-described NK cell exhaustion checkpoint in individuals with severe COVID-19 infection (Antonioli *et al.* 2020). Further, NK cell frequencies are restored

together with significant reduction in NKG2A in convalescing individuals suggesting that functional compromise of NK cells do occur in severe SARS-CoV-2 infection.

It is intriguing that a sizeable majority of COVID-19 patients (both) globally are believed to remain asymptomatic (Day 2020; He *et al.* 2020; Qiu 2020). A study by Long and colleagues also found that asymptomatic individuals displayed poor immune response to SARS-CoV-2 infections compared to symptomatic individuals. Here, these individuals exhibited lower levels of 18 pro- and anti-inflammatory cytokines or mediators arguing for implications on immunity strategy and serological surveys in the general responses to COVID-19 infections (Long *et al.* 2020). Therefore, mild immune responses to SARS-CoV-2 may be beneficial to the host as this will not result in cytokine storm syndrome that culminates in exaggerated immune destruction of the airway parenchyma and stromal cells of the lungs. There also exists a possibility of at least a limited degree of protection from development of severe clinical COVID-19 resulting from antibody cross-reactivity and development of partial neutralizing IgG responses from past interaction of the host with other classical seasonal cold-causing coronaviruses 229E, HKU1, OC43 and NL63 likely attributing to sub-clinical and/or asymptomatic COVID-19 and it remains to be seen if antibody-dependent enhancement (ADE) contributes to COVID-19 severity.

CONCLUSIONS AND PROSPECTS

To conclude, mechanisms uncovered in exotic mammals likely hint the development of therapeutic strategies against COVID-19 in humans. Given that asymptomatic transmission is increasingly becoming common in the current COVID-19 outbreak, this could also emerge as a significant public health challenge. Understanding the mechanisms associated with asymptomatic carriage of SARS-CoV-2 or existing defective innate viral sensing systems needs to be identified to predict asymptomatic carriage of the virus. Given that asymptomatic individuals continue to remain as 'ambassadors' of 'viral shedding' into the environment attributing to increased rates of viral transmission, it is imperative to urgently adopt out-of-the-box thinking strategies to curtail the rapid spread of COVID-19 in the community.

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Conflicts of Interest. None declared.

REFERENCES

Ahn M, Anderson DE, Zhang Q *et al.* Dampened NLRP3-mediated inflammation in bats and implications for a special viral reservoir host. *Nat Microbiol* 2019;**4**:789–99.

Andersen KG, Rambaut A, Lipkin WI *et al.* The proximal origin of SARS-CoV-2. *Nat Med* 2020;**26**:450–52.

Antonioli L, Fornai M, Pellegrini C *et al.* NKG2A and COVID-19: another brick in the wall. *Cell Mol Immunol* 2020;1–3. DOI: 10.1038/s41423-020-0450-7.

Bar-On YM, Flamholz A, Phillips R *et al.* SARS-CoV-2 (COVID-19) by the numbers. *eLife* 2020;**9**:e57309.

Brook CE, Boots M, Chandran K *et al.* Accelerated viral dynamics in bat cell lines, with implications for zoonotic emergence. *eLife* 2020;**9**:e48401.

Choo SW, Rayko M, Tan TK *et al.* Pangolin genomes and the evolution of mammalian scales and immunity. *Genome Res* 2016;**26**:1312–22.

Day M. Covid-19: identifying and isolating asymptomatic people helped eliminate virus in Italian village. *BMJ* 2020;**368**:m1165.

Diao B, Wang C, Tan Y *et al.* Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol* 2020;**11**:827.

Du Z, Sharma SK, Spellman S *et al.* KIR2DL5 alleles mark certain combination of activating KIR genes. *Genes Immun* 2008;**9**:470–80.

Fischer H, Tschachler E, Eckhart L. Cytosolic DNA sensing through cGAS and STING is inactivated by gene mutations in pangolins. *Apoptosis* 2020a;**25**:474–80.

Fischer H, Tschachler E, Eckhart L. Pangolins lack IFIH1 /MDA5, a cytoplasmic RNA sensor that initiates innate immune defense upon coronavirus infection. *Front Immunol* 2020b;**11**:939.

Giamarellos-Bourboulis EJ, Netea MG, Rovina N *et al.* Complex immune dysregulation in COVID-19 patients with severe respiratory failure. *Cell Host Microbe* 2020;**S1931-3128**:30236–5.

Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-induced hyperinflammation magnify the severity of coronavirus disease (CoViD-19) leading to acute respiratory distress syndrome? *Front Immunol* 2020;**11**:1206.

Guan WJ, Ni ZY, Hu Y *et al.* Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;**382**:1708–20.

He X, Lau EHY, Wu P *et al.* Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* 2020;**26**:672–5.

Ji W, Wang W, Zhao X *et al.* Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol* 2020;**92**:433–40.

Kacprzyk J, Hughes GM, Palsson-McDermott EM *et al.* A potent anti-inflammatory response in bat macrophages may be linked to extended longevity and viral tolerance. *Acta Chiropt* 2017;**19**:219–28.

Laing ED, Sterling SL, Weir DL *et al.* Enhanced autophagy contributes to reduced viral infection in black flying fox cells. *Viruses* 2019;**11**:260.

Lauer SA, Grantz KH, Bi Q *et al.* The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med* 2020; DOI: 10.7326/M20-0504.

Long QX, Tang XJ, Shi QL *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020.

Masselli E, Vaccarezza M, Carubbi C *et al.* A double edge sword against SARS-CoV-2. *Adv Biol Regulation* 2020;**77**:100737, DOI: 10.1016/j.jbior.2020.100737.

Mizumoto K, Kagaya K, Zarebski A *et al.* Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill* 2020;**25**: 2000180.

Our World in Data. India: coronavirus pandemic country profile, 2020, https ://ourworldindata.org/coronavirus/country/india?country=~IND.

Papenfuss AT, Baker ML, Feng Z-P *et al.* The immune gene repertoire of an important viral reservoir, the Australian black flying fox. *BMC Genomics* 2012;**13**: 261.

Qiu J. Covert coronavirus infections could be seeding new outbreaks. *Nature* 2020, DOI: 10.1038/d41586-020-00822-x.

Rivett L, Sridhar S, Sparkes D *et al.* Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission. *eLife* 2020;**9**: e58728.

Sharma V, Walther F, Hecker N *et al.* Convergent losses of TLR5 suggest altered extracellular flagellin detection in four mammalian lineages. *Mol Biol Evol* 2020, DOI: 10.1093/molbev/msaa058 26.

Shaw TI, Srivastava A, Chou W-C *et al.* Transcriptome sequencing and annotation for the Jamaican fruit bat (*Artibeus jamaicensis*). *PLoS One* 2012;**7**:e48472.

Tindale LC, Stockdale JE, Coombe M *et al.* Evidence for transmission of COVID-19 prior to symptom onset. *eLife* 2020;**9**: e57149.

Wang W, Xu Y, Ruqin G *et al.* Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 2020, DOI: 10.1001/jama.2020.3786.

Wei WE, Li Z, Chiew CJ *et al.* Presymptomatic transmission of SARS-CoV-2 — Singapore, January 23–March 16, 2020. MMWR, 2020;**69**, 411–415.

WHO Corona virus disease (COVID-19) Dashboard, 2020, https ://covid19.who.int/?gclid=Cj0KCQjw59n8BRD2ARIsAAmgPmKo1_Yp7FfVVffB-64cjTTRozIgGwZWTwqWNpjakwuUM3N75M2uV48aAtT_EALw_wcB.

WHO Situation Report Coronavirus (COVID-19) disease situation report - 73, 2020, https ://www.who.int/docs/default-source/coronaviruse/situation-reports/20200402-sitrep-73-Covid-19.pdf#:~:text=An%20asymptomatic%20laboratory%2Dconfirmed,does%20not%20develop%20symptoms.

Wu F, Zhao S, Yu B *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* 2020;**579**:265–9.

Zhang G, Cowled C, Shi Z *et al.* Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* 2013;**339**:456–60.

Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. *Curr Biol* 2020;**30**:1346–51.

Zheng J. SARS-CoV-2: an emerging coronavirus that causes a global threat. *Int J Biol Sci* 2020;**16**:1678–85.

Zheng M, Gao Y, Wang G *et al.* Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol* 2020;**17**:533–5.

Zhou P, Yang XL, Wang XG *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–3.

Zhu N, Zhang D, Wang W *et al.* A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;**382**: 727–33.