Summary

The novel coronavirus SARS-CoV-2 became pandemic at the beginning of 2020, and caused about 80 million cases and more than 1.8 million deaths by the end of the year. As its relatives MERS- and SARS-CoV, but in contrast to the four human coronaviruses circulating worldwide, SARS-CoV-2 in a sizeable fraction of cases leads to a severe and potentially life-threatening disease, called COVID-19. Since in addition this virus is very contagious, particularly prior to onset or in absence of symptoms, and pre-existing immunity appears to be largely absent or at least of very little relevance, it is spreading rapidly in the population. A hallmark of COVID-19 is an at least partially detrimental immune response that not only can lead to serious lung damage, but may also damage organs outside the respiratory tract such as the heart and kidneys. This review summarizes current knowledge about the virus and the disease it causes, and outlines open questions in the different research fields.

Keywords: SARS-CoV-2, COVID-19, molecular biology, transmission, epidemiology, pathogenesis, treatment, immunity

Zusammenfassung


Stichwörter: SARS-CoV-2, COVID-19, Molekularbiologie, Übertragung, Epidemiologie, Pathogenese, Therapie, Immunität
Introduction

The first human Coronaviruses (hCoVs) have been isolated some 50 years ago from individuals suffering from mild respiratory infections. Four such “common cold” coronaviruses are known to circulate worldwide in humans, namely hCoV-NL63, -HKU1, -229E, and -OC43 (Almeida and Tyrrell 1967, Fehr and Perlman 2015, Masters and Perlman 2013). In stark contrast, two zoonotic coronaviruses infecting humans and capable of spreading in the human population have been described to cause severe to life-threatening diseases: severe acute respiratory syndrome coronavirus (SARS-CoV), first emerging in 2002 and rapidly spreading to several countries around the globe (covered in detail by A. Osterhaus in this volume); and middle-east respiratory syndrome coronavirus (MERS-CoV), first described in 2012 (covered in detail by Asisa Volz in this volume). While circulation of SARS-CoV could be successfully contained by globally implemented public health measures, cases of MERS-CoV infection are still regularly reported, mainly on the Arabian peninsula (World Health Organization 2020a).

In December 2019, cases of severe pneumonia of unknown – but likely infectious – etiology in the city of Wuhan, China, have been reported to the WHO (World Health Organization 2020c), which were shortly after linked to a newly isolated coronavirus, first termed 2019-nCoV (World Health Organization 2020b). Due to its high similarity to SARS-CoV, the virus was later renamed SARS-CoV-2. However, as highlighted in other articles within this volume, coronaviruses have been identified and isolated from a large range of other mammalian species, particularly bats. In fact, RaTG13, a coronavirus found in a horseshoe bat in Yunnan province, China, exhibits an even higher degree of similarity than SARS-CoV to the newly emerged SARS-CoV-2 with approximately 96% of sequence identity at the nucleotide level throughout most genomic regions. It therefore appears likely that SARS-CoV-2 originated from a zoonotic event, either directly from bat or via an intermediate host (Andersen et al. 2020; Boni et al. 2020). Backing this hypothesis, the virus’ potential to readily cross species barriers was demonstrated by documented cases of SARS-CoV-2 transmission from humans to household animals (Shi et al. 2020; Sit et al. 2020) and from humans to farmed mink and back to humans (Oude Munnink et al. 2020). From lab experiments, it is known that particularly changes in the spike protein on the surface of the virions can lead to interspecies adaption in coronaviruses (Baric et al. 1997).

While the exact origin of SARS-CoV-2 still is under investigation, its impact on the human population has been dramatic. Since its first outbreak in Wuhan, it has spread with remarkable pace around the world, causing upwards of 80 million reported cases by the end of 2020, with more than 1.8 million deaths (World Health Organization 2021), and still raging. With SARS-CoV-2 and the associated coronavirus disease 2019 (COVID-19), human society thus faces a pandemic unbeknownst to living generations, with a broad spectrum of challenges for all disciplines of science. This article covers some of the key questions from the various branches of biomedical research.

Molecular biology of SARS-CoV-2 infection

Decades of research into the other coronaviruses have set the stage for fast progress in investigating SARS-CoV-2. As the general organization of the genes, detailed below, is conserved among all coronaviruses, SARS-CoV-2 was rapidly deciphered and viral protein coding genes were quickly defined. Furthermore, most aspects of the viral molecular lifecycle are shared with other coronaviruses (comprehensively reviewed in (VKovski et al. 2020)). As a plus-strand RNA virus, its genome constitutes a messenger RNA for the direct translation of viral proteins. Released from incoming virions into the cytosol right after cell entry, the viral genome serves as the message to produce viral proteins, which are translated as two long polyprotein precursors pp1a and pp1ab. The two polyproteins are then proteolytically cleaved by the viral cysteine protease, non-structural protein nsp3 (PLpro) and nsp5 (3CLpro), giving rise to the 16 nsps of the virus. These provide a multitude of enzymatic activities required for viral genome replication and the modulation of host cellular processes. One essential enzymatic function not present in the human genome is the RNA-dependent RNA polymerase (RdRp), comprising nsp12, nsp8 and nsp7, which is also the target of the direct acting antiviral (DAA) remdesivir (Sheahan et al. 2020). The RdRp then, through negative-strand intermediates, generates copies of the full-length genomic RNA as well as eight species of subgenomic mRNAs (sgmRNAs). Those sgRNAs code for structural proteins including nucleocapsid (N), spike (S), envelope (E) and membrane (M), as well as numerous auxiliary proteins. From other coronaviruses, in particular SARS-CoV, it is known that some of them antagonize host cellular antiviral defense pathways, most prominently the induction of and signaling by type I and III interferons (Park and Iwasaki 2020). Also, SARS-CoV-2 has been shown to encode an ample variety of activities targeting the host interferon and cytokine system (Hayn et al. 2020, Lei et al. 2020), believed to play a central role in dysregulating the immune response to infection and thereby crucially contributing to the development of COVID-19 (Achara et al. 2020, Mathew et al. 2020, McKechnie and Blish 2020).

From the structural proteins together with genomic RNA, new virions are formed by budding into membranous structures at the interface between the endoplasmic reticulum (ER) and the Golgi apparatus, likely representing the ER-Golgi-intermediate compartment (ERGIC), coherent with previous reports on other coronaviruses (Cortese et al. 2020, Fehr and Perlman 2015, Masters and Perlman 2013). Offspring virus particles are then released through exocytic processes, not strictly requiring cell lysis.

Released viral particles will then infect neighboring cells. Again owing to the close similarity to related viruses, including SARS-CoV, angiotensin converting enzyme 2 (ACE2), which plays a major role in regulating electrolyte and fluid balance as well as blood pressure (Verano-Braga et al. 2020), was readily identified as the major receptor for cell entry (Hoffmann et al. 2020). Alternative entry receptors, such as CD147 or NRP1, are currently under discussion (Cantuti-Castelvetri et al. 2020, Daly et al. 2020, Shills and Wright 2020, Wang et al. 2020a). It was further confirmed that the SARS-CoV-2
spike protein requires proteolytic processing (“priming”) to mediate entry, and that this priming can be efficiently mediated by the cellular membrane-bound TMPRSS2 protease (Hoffmann et al. 2020). TMPRSS2 as well as further cellular proteases shown to play alternative or redundant roles, including furin and cathepsin L, can be pharmacologically targeted to prevent infection (Hoffmann et al. 2020, Pislar et al. 2020, Shang et al. 2020).

**Transmission and epidemiology of SARS-CoV-2**

In contrast to many animal coronaviruses, all known hCoVs primarily infect and replicate in cells of the respiratory tract, and SARS-CoV-2 is no exception in this regard (Fehr and Perlman 2015, Masters and Perlman 2013, Salzberger et al. 2020). In contrast to SARS-CoV, mostly replicating in epithelial cells of the lower respiratory tract, SARS-CoV-2 was found to infect and efficiently replicate also in cells of the upper respiratory tract, with nasopharyngeal and oropharyngeal swabs but also saliva containing high amounts of viral RNA and infectious virus (Cevik et al. 2020a, Wolfel et al. 2020, Wylie et al. 2020). As a consequence, SARS-CoV-2 can easily transmit upon coughing, sneezing, singing and even talking. These activities produce a spray of droplets ranging from visible, large droplets, which will fall to the ground relatively quickly compared to microscopic (< 100 μM) (Prather et al. 2020) droplet nuclei lingering in the air as aerosols (Fennelly 2020, Klompas et al. 2020, van Doremalen et al. 2020). While transmission by larger droplets appears more prevalent, long distance transmission by aerosols may, at least in part, explain so-called “superspreading events”, in which a small number of index patients, or even only a single infected person, transmit the virus to large numbers of people in the same confined space (Cevik et al. 2020a), for example in large open-plan offices (Park et al. 2020b), festivities (Ghina et al. 2020), churches (James et al. 2020), meat processing facilities (Guenter et al. 2020) or choirs (Hamner et al. 2020). Similar to SARS-CoV, but different from most seasonal respiratory viral infections, the higher propensity of SARS-CoV-2 to transmit via such one-to-many “superspreading events” needs to be taken into account for epidemiological modelling. The number of secondary cases caused by single patients is generally very low, between zero and one for most patients, but follows a negative binomial distribution with a very long tail, corresponding to an average R0 between 2 and 3 with a small (much less than 1) dispersion parameter (Adam et al. 2020, Althouse et al. 2020, Kupferschmidt 2020, Li et al. 2020a, Liu et al. 2020b, Park et al. 2020a).

Another feature of SARS-CoV-2 transmission is pre-symptomatic contagiousness, and possibly transmission by infectees who remain fully asymptomatic. While the extent of the contribution of truly asymptomatic carriers to the overall epidemiology of SARS-CoV-2 is still debated, transmission one to two days prior to the onset of symptoms likely plays a decisive role in rapid community spread (Cevik et al. 2020a, Kasper et al. 2020). In general, transmissibility is highest around the onset of symptoms and quickly declining thereafter (Cevik et al. 2020b).

Taking those basic epidemiological characteristics into account, policy responses at the beginning of the pandemic early on prohibited large gatherings and especially indoor events with large numbers of attendees, and in a second step, many countries promoted the use of face masks to curb virus spread (Flaxman et al. 2020). By and large, these public health measures were shown to be actually effective (Chu et al. 2020, Dehning et al. 2020). However, epidemiological analyses are still ongoing, as the situation is highly complex and differs between regions, countries and continents. In general, human respiratory viruses and in particular the endemic hCoVs exhibit clear seasonal profiles (Moriyama et al. 2020). In fact, Europe experienced a low-incidence situation throughout summer, but suffered from steeply increasing case numbers from September/October on, reaching multiples of the numbers recorded in spring (Cacciapaglia et al. 2020, European Centre for Disease Prevention and Control 2020, Looi 2020).

The use of high-throughput RNA sequencing to determine the entire sequence of patient isolates, although varying between countries, has identified a myriad of sequence variants, which are deposited and available through database platforms such as NextStrain (Hadfield et al. 2018). Phylogenetic analyses of viral sequences enable close tracking of the mutational course of the virus. This not only permits the precise reconstruction of transmission chains and epidemiologic events (Pupa et al. 2020), but also allows for worldwide monitoring of the genomic landscape of SARS-CoV-2 and the identification of mutations that got fixed in the viral genome. Mutations within the S protein of circulating viruses are particularly in focus, as it is both the major determinant for efficient cell entry and the major antigen for neutralizing antibody responses. Also mutations in other parts of the genome might confer increased virulence. Of note, coronaviruses generally have relatively low mutation rates as they uniquely bear a proofreading replication mechanism, likely due to their genomes being amongst the largest (~30,000 bases) of all RNA viruses (Fehr and Perlman 2015, Masters and Perlman 2013).

An early spike protein mutation, D614G, emerged within the first weeks of the pandemic and quickly took over (Korber et al. 2020). In fact, it could be demonstrated that the D614G variant shifts the S protein conformation towards higher ACE2-binding and fusogenicity, leading to increased infectivity of the virus in vitro (Korber et al. 2020, Yurkovetskiy et al. 2020). Recently, it was confirmed that also in vivo transmissibility in animal models was significantly enhanced in the D614G variant, however, pathogenicity was not (Hou et al. 2020a). In December 2020, a new variant (B1.1.7) bearing several mutations in the S protein was identified in the United Kingdom, which was spreading rapidly, again indicating significantly higher transmissibility (Davies et al. 2020).

**Clinical characteristics of COVID-19**

Respiratory viruses are very common. Rhinoviruses, adenoviruses, respiratory syncytial viruses and of course influenza- and coronaviruses, cause a range of illnesses, including the very frequent common cold. SARS-CoV-2 was initially described to cause severe viral pneumonia, resembling the clinical picture of SARS and MERS, displaying typical ground-glass-opacities in chest radiography and leading to acute respiratory distress syndrome (ARDS) in a sizeable proportion of patients (Huang et al. 2020).
COVID-19 pathogenesis and treatment options

The pathomechanisms underlying this very broad spectrum of symptoms are not yet understood. An emerging general view is that the disease proceeds in two phases (Li et al. 2020b, Tay et al. 2020). In the first phase, the virus infects and replicates in epithelial cells of the upper respiratory tract. It then spreads and descends until it reaches cells in the lung, likely alveolar epithelial cells. During this phase, viral infection induces innate immune responses, recruiting macrophages and monocytes, but also cytotoxic cells to the sites of infection. It is likely that in the majority of patients suffering from mild symptoms, this regular immune response is sufficient to effectively control the virus.

However, in moderate to critical courses of the disease, a second phase with potentially life-threatening consequences initiates. The currently prevalent hypothesis focuses on the hyperactivation of immune cells and a massive secretion of cytokines, sometimes termed “cytokine storm” (Li et al. 2020b, Mangalmurti and Hunter 2020, Moore and June 2020, Tay et al. 2020). Although not fully understood, it appears likely that following infection, the cellular response is shifted away from antiviral interferons towards pro-inflammatory cytokine secretion (Acharya et al. 2020, Galani et al. 2021, Neufeldt et al. 2020).

Broadly speaking, the research on the causes of severe COVID-19 can be divided in two areas. First, there is the question of where and how does this detrimental response to the infection initiates. Second, factors that contain or amplify and worsen the outcome shall be identified.

To answer the first question, experiments are conducted in a range of model systems and combined with data from patient samples. An important limitation for the latter is that due to the lag time from infection to symptom onset and hospitalization, early stages of the infection cannot be monitored.

Initial studies in cell culture showed an induction of pro-inflammatory genes in virus-infected epithelial cells (Blanco-Melo 2020), however, as for interferon genes, only in small subsets of cells (Fiege et al. 2020, Wyler et al. 2020). Data from bronchoalveolar lavages (Liao et al. 2020), *in vivo* infected human lung tissue (Hönzke et al. 2020) or Syrian hamsters (Nouailles et al. 2020) indicate that, at least in the lower airways, infection of epithelial cells is neither particularly efficient, likely also due to low levels of ACE2 expression (Hönzke et al. 2020, Hou et al. 2020b), nor elicits a strong transcriptional response. However, these studies support an important role for macrophages in triggering a potentially excessive pro-inflammatory response. Along these lines, macrophages...
Immunity to SARS-CoV-2

For the four common cold hCoVs, current knowledge indicates that immunity is often partial and might not last longer than several months to a few years (Edridge et al. 2020). However, due to their widespread circulation, through recurrent infections antibody serum levels can remain elevated (Edridge et al. 2020). It has been proposed that a certain degree of immunity may be cross-protective between the different endemic hCoVs and in fact, a small percentage of SARS-CoV-2-naïve sera contained anti-hCoV-IgG capable of neutralizing SARS-CoV-2 (Ng et al. 2020). Along these lines, a certain cross-reactivity was also shown on the level of memory B cells (Sokal et al. 2021). A stronger and putatively longer lasting effect was seen in T-cell responses, where circulating T-lymphocytes specific to epitopes of endemic hCoVs proved to be cross-reactive to epitopes of SARS-CoV-2 (Braun et al. 2020, Le Bert et al. 2020, Mateus et al. 2020). Of note, these studies have shown cross-reactivity, however, it remains to be investigated if these responses would be protective (de Vries 2020), and even if so, it may not have substantial impact on the current epidemiological understanding of the pandemic (Lipsitch et al. 2020).

Whereas pre-existing immunity, as described above, likely has a very limited impact, a large majority of people infected with SARS-CoV-2 rapidly develops a robust and specific antibody and memory B cell response (Guo et al. 2020, Long et al. 2020, Okba et al. 2020, Sokal et al. 2021), with IgA dominating the early neutralizing activity (Sterlin et al. 2020). The induction of humoral immunity may depend on the severity of the disease (Roltgen et al. 2020), however, strong immune response were reported across all disease severities. Some reports indicate a rather quick waning of circulating antibodies (Bruni et al. 2020, Roltgen et al. 2020, Seow et al. 2020), whereas others suggest that protective antibody titers may remain robustly elevated at least for five months (Gudbjartsson et al. 2020, Wajnberg et al. 2020), and B-cell memory likely persists for even longer (Rodda et al. 2020, Sokal et al. 2020). Treatment of severe COVID-19 cases with plasma of convalescent patients has been proposed and clinically tested, but yielded mixed results (Liu et al. 2020a, Rojas et al. 2020, Simonovich et al. 2020).

Beside antibody responses, also T-cell immunity is induced upon infection with SARS-CoV-2 (Cox and Brokstad 2020). While T-cells may contribute to immunopathology in COVID-19 (Gustine and Jones 2021, Mathew et al. 2020, Yang et al. 2020b), they may also play an important role in controlling the infection and, possibly, mediating long-term protection (Chen and John Wherry 2020, Chen and John Wherry 2020, Cox and Brokstad 2020, Le Bert et al. 2020, Rydyznyski Moderbacher et al. 2020). As it is difficult to functionally relate reactivity to actual protection, the contributions of CD4+ and CD8+ T-lymphocytes requires further investigation. Understanding their role in COVID-19 better, should further direct the development of vaccine candidates.

Owing to the unprecedented importance of controlling this novel viral threat, more than 180 vaccine candidates are currently under development ([Krammer 2020]; see also the article by M. Bastian in this volume). Preliminary reports were insofar promising as they report very strong immune responses across a range of vaccine types (Folegatti et al. 2020, Jackson et al. 2020, Keech et al. 2020, Krammer 2020, Mulligan et al. 2020, Zhu et al. 2020). Two vaccines, both first-of-their-kind mRNA-based approaches (Baden et al. 2020, Polack et al. 2020), and two adenovirus-vectorized vaccine candidates showed efficacies in phase 3 clinical studies ranging up to about 90% (Logunov et al. 2020, Voysey et al. 2020). Around the globe, more and more vaccines with different methodologies (including inactivated viruses and purified antigens) are being established and approved. Broad vaccinations that likely offer a high degree of protection at least for a limited period of time, thus, appear to be in feasible reach. If necessary, regular overhauls of the vaccines due to a mutating
virus, somewhat akin to the current influenza vaccination approach, is quickly possible at least with non-vectored vaccines and opens the chance for sustained control of the SARS-CoV-2 pandemic.

**Summary and outlook**

The year 2020 has not only seen a rapid spread of the novel coronavirus SARS-CoV-2, but also a massive and worldwide effort in all fields of biomedical research and beyond. Owing to substantial progress in elucidating the basic biology of virus infection and the molecular pathogenesis of severe COVID-19, possible paths to reliable treatments are only slowly clearing up. In contrast, a broad range of vaccines shown to be safe and efficacious were developed and approved with unprecedented speed. Challenges for the months and years to come include, among others, the elucidation of the frequency, causes and severity of long-term health impairments, and to translate the accumulating knowledge about SARS-CoV-2 biology into therapeutic strategies. With the prospect of a quickly increasing share of the world’s population being vaccinated, the surveillance of possibly emerging vaccine-resistant mutants will further be essential for a sustainable control of SARS-CoV-2.

**Ethical Approval**

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**Conflict of interest**

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