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challenge with MERS-CoV (169). The intranasal administration of the recombinant adenovirus-based vaccine in BALB/c mice was found to induce long-lasting neutralizing immunity against MERS spike pseudotyped virus, characterized by the induction of systemic IgG, secretory IgA, and lung-resident memory T-cell responses (177). Immunoinformatics methods have been employed for the genome-wide screening of potential vaccine targets among the different immunogens of MERS-CoV (178). The N protein and the potential B-cell epitopes of MERS-CoV E protein have been suggested as immunoprotective targets inducing both T-cell and neutralizing antibody responses (178, 179).

The collaborative effort of the researchers of Rocky Mountain Laboratories and Oxford University is designing a chimpanzee adenovirus-vectored vaccine to counter COVID-19 (180). The Coalition for Epidemic Preparedness Innovations (CEPI) has initiated three programs to design SARS-CoV-2 vaccines (181). CEPI has a collaborative project with Inovio for designing a MERS-CoV DNA vaccine that could potentiate effective immunity. CEPI and the University of Queensland are designing a molecular clamp vaccine platform for MERS-CoV and other pathogens, which could assist in the easier identification of antigens by the immune system (181). CEPI has also funded Moderna to develop

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appeared asymptomatic[45]. Another serological study detected SARS-CoV-2 neutralizing antibodies in cat serum samples collected in Wuhan after the COVID-19 outbreak, providing evidence for SARS-CoV-2 infectionin cat populations in Wuhan, although the potential of SARS-CoV-2 transmission from cats to humans is currently uncertain[46].

Receptor use and pathogenesis

SARS-CoV-2 uses the same receptor as SARS-CoV, angiotensin-converting enzyme 2 (ACE2)[11,47]. Besides human ACE2 (hACE2), SARS-CoV-2 also recognizes ACE2 from pig, ferret, rhesus monkey, civet, cat, pan-golin, rabbit and dog[11,43,48,49]. The broad receptor usage of SARS-CoV-2 implies that it may have a wide host range, and the varied efficiency of ACE2 usage in different animals may indicate their different susceptibilities to SARS-CoV-2 infection. The S1 subunit of a coronavirus is further divided into two functional domains, an N-terminal domain and a C-terminal domain.
Structural and biochemical analyses identified a 211 amino acid region (amino acids 319–529) at the S1C-terminal domain of SARSCoV-2 as the RBD, which has a key role in virus entry and is the target of neutralizing antibodies[50,51] (FIG. 3a). The RBD mediates contact with the ACE2 receptor (amino acids 437–507 of SARS-CoV-2 S protein), and this region in SARS-CoV-2 differs from that in SARS-CoV in the five residues cr-

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fever, cough, and sputum (83). Hence, the clinicians must be on the look-out for the possible occurrence of atypical clinical manifestations to avoid the possibility of missed diagnosis. The early transmission ability of SARS-CoV-2 was found to be similar to or slightly higher than that of SARS-CoV, reflecting that it could be controlled despite moderate to high transmissibility (84).

Increasing reports of SARS-CoV-2 in sewage and wastewater warrants the need for further investigation due to the possibility of fecal-oral transmission. SARS-CoV-2 present in environmental compartments such as soil and water will finally end up in the wastewater and sewage sludge of treatment plants (328). Therefore, we have to reevaluate the current wastewater and sewage sludge treatment procedures and introduce advanced techniques that are specific and effective against SARS-CoV-2. Since there is active shedding of SARS-CoV-2 in the stool, the prevalence of infections in a large population can be studied using wastewater-based epidemiology. Recently, reverse transcription-quantitative PCR (RT-qPCR) was used to enumerate the copies of SARS-CoV-2 RNA concentrated from wastewater collected from a wastewater treatment plant (327). The calculated viral RNA copy numbers determine the number of infected individuals. The

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explored targeting molecular dynamic simulations, evaluating their interaction with corresponding major histocompatibility complex class I molecules. They potentially induce immune responses (176). The recombinant vaccine can be designed by using rabies virus (RV) as a viral vector. RV can be made to express MERS-CoV S1 protein on its surface so that an immune response is induced against MERS-CoV. The RV vector-based vaccines against MERS-CoV can induce faster antibody response as well as higher degrees of cellular immunity than the Gram-positive enhancer matrix (GEM) particle vaccine vector-based vaccine. However, the latter can induce a very high antibody response at lower doses (167). Hence, the degree of humoral and cellular immune responses produced by such vaccines depends upon the vector used.

Dual vaccines have been getting more popular recently. Among them, the rabies virus-based vectored vaccine platform is used to develop vaccines against emerging infectious diseases. The dual vaccine developed from inactivated rabies virus particles that express the MERS-CoV S1 domain of S protein was found to induce immune responses for both MERS-CoV and rabies virus. The vaccinated mice were found to be completely protected from challenge with MERS-CoV (169). The intranasal

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specimens, like bronchoalveolar lavage fluid, sputum, nasal swabs, fibrobronchoscope brush biopsy specimens, pharyngeal swabs, feces, and blood (246).

The presence of SARS-CoV-2 in fecal samples has posed grave public health concerns. In addition to the direct transmission mainly occurring via droplets of sneezing and coughing, other routes, such as fecal excretion and environmental and fomite contamination, are contributing to SARS-CoV-2 transmission and spread (249–252). Fecal excretion has also been documented for SARS-CoV and MERS-CoV, along with the potential to stay viable in situations aiding fecal-oral transmission. Thus, SARS-CoV-2 has every possibility to be transmitted through this mode. Fecal-oral transmission of SARS-CoV-2, particularly in regions having low standards of hygiene and poor sanitation, may have grave consequences with regard to the high spread of this virus. Ethanol and disinfectants containing chlorine or bleach are effective against coronaviruses (249–252). Appropriate precautions need to be followed strictly while handling the stools of patients infected with SARS-CoV-2. Biowaste materials and sewage from hospitals must be adequately disinfected, treated, and disposed of properly. The significance of frequent and good hand hygiene and

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Currently, our knowledge on the animal origin of SARS-CoV-2 remains incomplete to a large part. The reservoir hosts of the virus have not been clearly proven. It is unknown whether SARS-CoV-2 was transmitted to humans through an intermediate host and which animals may act as its intermediate host. Detection of RaTG13, RmYN02 and pangolin coronaviruses implies that diverse coronaviruses similar to SARS-CoV-2 are circulating in wildlife. In addition, as previous studies showed recombination as the potential origin of some sarbecoviruses such as SARS-CoV, it cannot be excluded that viral RNA recombination among different related coronaviruses was involved in the evolution of SARS-CoV-2. Extensive surveillance of SARS-CoV-2-related viruses in China, Southeast Asia and other regions targeting bats, wild and captured pangolins and other wildlife species will help us to better understand the zoonotic origin of SARS-CoV-2.

Besides wildlife, researchers investigated the susceptibility of domesticated and laboratory animals to SARS-CoV-2 infection. The study demonstrated experimentally that SARS-CoV-2 replicates efficiently in cats and in the upper respiratory tract of ferrets, whereas dogs, pigs, chickens and ducks were not susceptible to SARS-CoV-2 (REF.[43]). The susceptibility of minks was documented by a report from the Netherlands on an outbreak of SARS-CoV-2 infection in farmed minks. Although the symptoms in most infected minks were mild, some developed severe respiratory distress and died of interstitial pneumonia[44]. Both virological and serological testing found transmission of SARS-CoV-2 infection in two dogs from households with human cases of COVID-19 in Hong Kong, but the dogs

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proteins without the presence of S protein would not confer any noticeable protection, with the absence of detectable serum SARS-CoV-neutralizing antibodies (170). Antigenic determinant sites present over S and N structural proteins of SARS-CoV-2 can be explored as suitable vaccine candidates (294). In the Asian population, S, E, M, and N proteins of SARS-CoV-2 are being targeted for developing subunit vaccines against COVID-19 (295).

The identification of the immunodominant region among the subunits and domains of S protein is critical for developing an effective vaccine against the coronavirus. The C-terminal domain of the S1 subunit is considered the immunodominant region of the porcine deltacoronavirus S protein (171). Similarly, further investigations are needed to determine the immunodominant regions of SARS-CoV-2 for facilitating vaccine development.

However, our previous attempts to develop a universal vaccine that is effective for both SARS-CoV and MERS-CoV based on T-cell epitope similarity pointed out the possibility of cross-reactivity among coronaviruses (172). That can be made possible by selected potential vaccine targets that are common to both viruses. SARS-CoV-2 has been reported to be closely related to SARS-CoV (173, 174). Hence, knowledge and understanding of

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The exploration of fully human antibodies (human single-chain antibodies; HuscFvs) or humanized nanobodies (single-domain antibodies; sdAb, VH/VHH) could aid in blocking virus replication, as these agents can traverse the virus-infected cell membranes (transbodies) and can interfere with the biological characteristics of the replicating virus proteins. Such examples include transbodies to the influenza virus, hepatitis C virus, Ebola virus, and dengue virus (206). Producing similar transbodies against intracellular proteins of coronaviruses, such as papain-like proteases (PLpro), cysteine-like protease (3CLpro), or other nsps, which are essential for replication and transcription of the virus, might formulate a practical move forward for a safer and potent passive immunization approach for virus-exposed persons and rendering therapy to infected patients.

In a case study on five grimly sick patients having symptoms of severe pneumonia due to COVID-19, convalescent plasma administration was found to be helpful in patients recovering successfully. The convalescent plasma containing a SARS-CoV-2-specific ELISA (serum) antibody titer higher than 1:1,000 and neutralizing antibody titer more significant than 40 was collected from the recovered patients and used for plasma transfusion

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another study, the average reproductive number of COVID-19 was found to be 3.28, which is significantly higher than the initial WHO estimate of 1.4 to 2.5 (77). It is too early to obtain the exact R_0 value, since there is a possibility of bias due to insufficient data. The higher R_0 value is indicative of the more significant potential of SARS-CoV-2 transmission in a susceptible population. This is not the first time where the culinary practices of China have been blamed for the origin of novel coronavirus infection in humans. Previously, the animals present in the live-animal market were identified to be the intermediate hosts of the SARS outbreak in China (78). Several wildlife species were found to harbor potentially evolving coronavirus strains that can overcome the species barrier (79). One of the main principles of Chinese food culture is that live-slaughtered animals are considered more nutritious (5).

After 4 months of struggle that lasted from December 2019 to March 2020, the COVID-19 situation now seems under control in China. The wet animal markets have reopened, and people have started buying bats, dogs, cats, birds, scorpions, badgers, rabbits, pangolins (scaly anteaters), minks, soup from palm civet, ostriches, hamsters, snapping turtles, ducks, fish, Siamese crocodiles, and other

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fatigue. Individuals with asymptomatic and atypical clinical manifestations were also identified recently, further adding to the complexity of disease transmission dynamics. Atypical clinical manifestations may only express symptoms such as fatigue instead of respiratory signs such as fever, cough, and sputum. In such cases, the clinician must be vigilant for the possible occurrence of asymptomatic and atypical clinical manifestations to avoid the possibility of missed diagnoses.

The present outbreak caused by SARS-CoV-2 was, indeed, expected. Similar to previous outbreaks, the current pandemic also will be contained shortly. However, the real question is, how are we planning to counter the next zoonotic CoV epidemic that is likely to occur within the next 5 to 10 years or perhaps sooner? Our knowledge of most of the bat CoVs is scarce, as these viruses have not been isolated and studied, and extensive studies on such viruses are typically only conducted when they are associated with specific disease outbreaks. The next step following the control of the COVID-19 outbreak in China should be focused on screening, identification, isolation, and characterization of CoVs present in wildlife species of China, particularly in bats. Both in vitro and in vivo studies (using suitable animal models) should be conducted

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category A agents (cholera, plague). Patients should be placed in separate rooms or cohorted together. Negative pressure rooms are not generally needed. The rooms and surfaces and equipment should undergo regular decontamination preferably with sodium hypochlorite. Healthcare workers should be provided with fit tested N95 respirators and protective suits and goggles. Airborne transmission precautions should be taken during aerosol generating procedures such as intubation, suction and tracheostomies. All contacts including healthcare workers should be monitored for development of symptoms of COVID-19. Patients can be discharged from isolation once they are afebrile for atleast 3 d and have two consecutive negative molecular tests at 1 d sampling interval. This recommendation is different from pandemic flu where patients were

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patients with COVID-19 can be found on the WHO and CDC websites.[67]

16 CONCLUSION

The corona virus (COVID-19) spreads at an alarming rate all over the world. The outbreak of the virus has confronted the world's economic, medical and public health infrastructure. Elderly and immunocompromised patients also are susceptible to the virus's mortal impacts. Currently, there is no documented cure for the virus and no vaccine has been created, although some treatment protocols have been promising. Therefore, the virus can be controlled with the appropriate prevention strategies. Also, attempts have to be made to formulate systematic strategies to prevent such future zoonotic outbreaks.

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a polybasic cleavage site (RRAR), which enables effective cleavage by furin and other proteases[27]. Such an S1–S2 cleavage site is not observed in all related viruses belonging to the subgenus Sarbecovirus, except for a similar three amino acid insertion (PAA) in RmYN02, a bat-derived coronavirus newly reported from *Rhinolophus malayanus* in China[28] (FIG. 3a). Although the insertion in RmYN02 does not functionally represent a polybasic cleavage site, it provides support for the notion that this characteristic, initially considered unique to SARS-CoV-2, has been acquired naturally[28]. A structural study suggested that the furin-cleavage site can reduce the stability of SARS-CoV-2 S protein and facilitate the conformational adaptation that is required for the binding of the RBD to its receptor[29]. Whether the higher transmissibility of SARS-CoV-2 compared with SARS-CoV is a gain of function associated with acquisition of the furin-like cleavage site is yet to be demonstrated[26].

An additional distinction is the accessory gene *orf8* of SARS-CoV-2, which encodes a novel protein showing only 40% amino acid identity to ORF8 of SARS-CoV. Unlike in SARS-CoV, this new ORF8 protein does not contain a motif that triggers intracellular stress pathways[25]. Notably, a SARS-CoV-2 variant with a 382-nucleotide deletion covering the whole of ORF8 has been discovered in a number of patients in Singapore, which resembles the 29- or 415-nucleotide deletions in the ORF8 regions observed in human SARS-CoV variants from the late phase of the 2002–2003 outbreak[30]. Such ORF8 deletion variants seem to be involved in the fast across-species transmission from an animal host.

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therapeutics, and drug regimens to counter emerging viruses (161–163, 280). Several attempts are being made to design and develop vaccines for CoV infection, mostly by targeting the spike glycoprotein. Nevertheless, owing to extensive diversity in antigenic variants, cross-protection rendered by the vaccines is significantly limited, even within the strains of a phylogenetic subcluster (104). Due to the lack of effective antiviral therapy and vaccines in the present scenario, we need to depend solely on implementing effective infection control measures to lessen the risk of possible nosocomial transmission (68). Recently, the receptor for SARS-CoV-2 was established as the human angiotensin-converting enzyme 2 (hACE2), and the virus was found to enter the host cell mainly through endocytosis. It was also found that the major components that have a critical role in viral entry include PIKfyve, TPC2, and cathepsin L. These findings are critical, since the components described above might act as candidates for vaccines or therapeutic drugs against SARS-CoV-2 (293).

The majority of the treatment options and strategies that are being evaluated for SARS-CoV-2 (COVID-19) have been taken from our previous experiences in treating SARS-CoV, MERS-CoV, and other emerging viral diseases. Several therapeutic

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4 VIROLOGY

Coronaviruses, a family of viruses within the nidoviruses superfamily, are further classified according to their genera, alpha-, beta-, gamma- and deltacoronaviruses (α-, β-, γ- and δ-). Among those, alpha and beta species are capable of contaminating only mammals, whereas the other two genera can infect birds and could also infect mammals.[13,14] Two of these genera belong to human coronaviruses (HCoVs): α-coronaviruses, which comprise human coronavirus 229E (hcov229E) and human coronavirus NL63 (hcovNL63), and β-coronaviruses, which are human coronavirus HKU1, human coronavirus OC43, MERS-CoV (known as Middle East respiratory syndrome coronavirus) and SARS-CoV (referred to as severe acute respiratory syndrome coronavirus).[15]

The severe acute respiratory syndrome CoV-2 (SARS-CoV-2) is now named novel COVID-19 (coronavirus disease 2019).[16] Genome sequencing and phylogenetic research revealed that the COVID-19-causing coronavirus is a beta-coronavirus that belongs to the same subtypes as SARS virus, but represents a new variant group. The receptor-binding gene region