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REVIEW

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Immune responses and pathogenesis of SARS-CoV-2 during an outbreak in Iran: Comparison with SARS and MERS

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Summary

The beginning of 2020 has seen the emergence of COVID-19, an outbreak caused by a novel coronavirus, SARS-CoV-2, an important pathogen for humans. There is an urgent need to better understand this new virus and to develop ways to control its spread. In Iran, the first case of the COVID-19 was reported after spread from China and other countries. Fever, cough, and fatigue were the most common symptoms of this virus. In worldwide, the incubation period of COVID-19 was 3 to 7 days and approximately 80% of infections are mild or asymptomatic, 15% are severe, requiring oxygen, and 5% are critical infections, requiring ventilation. To mount an antiviral response, the innate immune system recognizes molecular structures that are produced by the invasion of the virus. COVID-19 infection induces IgG antibodies against N protein that can be detected by serum as early as day 4 after the onset of disease and with most patients seroconverting by day 14. Laboratory evidence of clinical patients showed that a specific T-cell response against SARS-CoV-2 is important for the recognition and killing of infected cells, particularly in the lungs of infected individuals. At present, there is no specific antiviral therapy for COVID-19 and the main treatments are supportive. In this review, we investigated the innate and acquired immune responses in patients who recovered from COVID-19, which could inform the design of prophylactic vaccines and immunotherapy for the future.

KEYWORDS
2019-nCoV disease, B cell, coronavirus, COVID-19, immunity, SARS-CoV-2, T cell

1 | INTRODUCTION

Coronaviruses are important pathogens for humans and livestock, birds, bat, mouse, and many other wild animals which can infect respiratory, gastrointestinal, hepatic, and central nervous systems of these vertebrates.[1]

Outbreaks that caused worldwide severe acute respiratory syndrome (SARS) in 2002-2003 and the Middle East Respiratory Syndrome (MERS) in 2012 demonstrated the possibility of animal-to-human and human-to-human transmission of newly emerging Coronaviruses. On 12 January 2020, the World Health Organization (WHO) named the latest virus as SARS-CoV-2 as the cause of 2019 novel coronavirus infectious disease (COVID-19).[2,3]

In late 2019, the first case of the COVID-19 was reported in Wuhan, a large city in China[4] and then in February 2020, COVID-19 was reported in Iran and other countries, and transmission rate, mortality rate and the clinical manifestation slowly emerged.[5] In Iran on 19 March 2020, a total of 18 407 confirmed cases were reported with an apparent fatality rate of 6.98% (Figure 1). As a result, approximately 0.02% of the Iranian population is affected by coronavirus disease. This index is currently at about 0.01% for China and less than 0.01% for Japan. However, Iran is less affected than Spain and Italy, according to official data from the WHO.

Based on data from previous coronaviruses, this review aims to fill the knowledge gap about the human immune response to SARS-CoV-2 infection that may help in designing the appropriate immune

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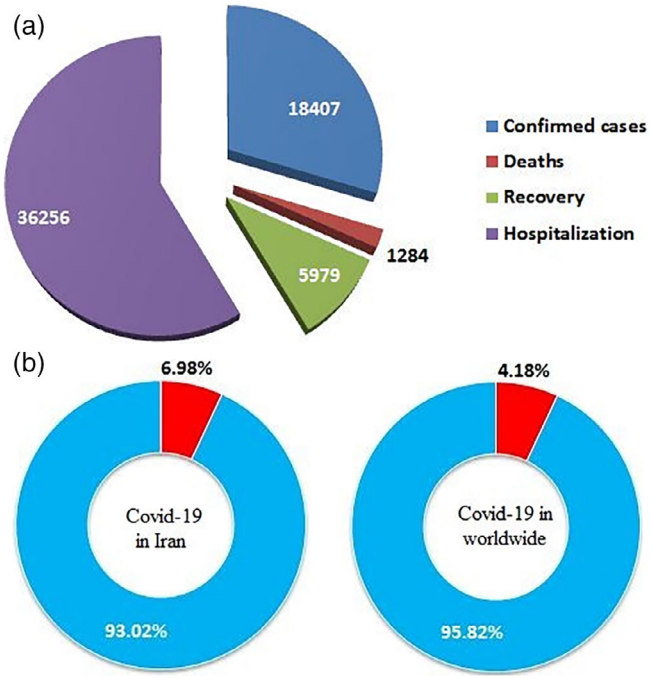

FIGURE 1 Comparative data of A, confirmed cases, deaths, recovery in Iran until 2020/19/03, and B, mortality rate of COVID-19 infection in Iran and other countries (Numbers on Venn diagram represent death/confirmed cases.)

intervention for treatment, diagnosis, and prophylactic/therapeutic vaccines against COVID-19.

2 | GENOME STRUCTURE OF SARS-COV-2

SARS-CoV-2 belongs to the family *Coronaviridae* of the *Nidovirales* order. The genome is positive-sense single-stranded RNA (+ssRNA) (~29.8 kbp) with 5'cap structure and 3'poly-A tail. The genomic RNA between ORF1a and ORF1b (open reading frames) is used for direct production of two polypeptides: polyprotein 1a/1ab (pp1a/pp1ab), which encodes non-structural proteins (nsps).[6] ORFs on the one-third of the genome near the 3'terminus encode at least four main structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins[6,7] (Figure 2A). The phylogenetic tree shows that the virus undergoes immunologic pressure and can increase the frequency of mutations in the genetic sequence of coronavirus among people from different countries. This process can increase viral virulence and transmissibility[8] (Figure 2B).

3 | IMMUNOPATHOLOGY OF COVID-19 DISEASE

In worldwide, based on hospitalized patient data, the incubation period of SARS-CoV-2 was 3 to 7 days and approximately 80% of infections are mild or asymptomatic, 15% are severe, requiring oxygen, and 5% are critical infections, requiring ventilation.[9] Fever, cough, and fatigue were the most common symptoms.[10] The spike protein receptor-binding domain (RBD) predicts that SARS-CoV-2 may use angiotensin-converting enzyme 2 (ACE2) as a host cell receptor for infection in human airway epithelia that is the most commonly damaged organ.[11,12] It is still unknown if SARS-CoV-2 infects any immune cells because only a small percentage of lung resident monocytes/macrophages express the ACE2 receptor. It is possible that other receptors may exist, or another cellular entry mode is utilized such as antibody-dependent enhancement.[13]

Many studies related to COVID-19 suggest a protective role of both cell-mediated and humoral immune responses in humans.[14,15] The S protein and the N protein of SARS-CoV-2 were the most immunogenic and abundantly expressed proteins during infection.[15]

In a study in China, most patients developed lymphopenia and pneumonia with characteristic pulmonary ground-glass opacity changes on a chest CT scan that was gold standard in diagnostic. Other changes that were usually observed in patients of this study included: lymphopenia (89.2%), neutrophilia (74.3%), and thrombocytopenia (24.3%). In addition, most patients had a high neutrophil-to-lymphocyte ratio of >5 (94.5%), high systemic immune-inflammation index of >500 (89.2%), increased C-reactive protein level (100%), lactate dehydrogenase (93.2%), and D-dimer (97.1%). A high level of IL-6 (>10 pg/mL) was observed in all detected patients.[16] The result of a study in hospitalized patients with high levels of proinflammatory cytokines including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα were detected in the severe infection called "cytokine storm" or cytokine release syndrome (CRS) as a major factor in the pathogenesis of COVID-19.[17,18]

4 | THE ROLE OF INNATE IMMUNE RESPONSES TO SARS-COV-2 INFECTION

Currently, only a few studies are available on the host innate immune response of COVID-19 infected patients. In one project in Buali hospital in Zahedan city of Iran (unpublished), 35 cases were assessed and increased total neutrophils (48%), reduced absolute lymphocyte count under 1100 μ/L (45%), increased serum IL-6 (58%), and increased C-reactive protein (99%) were detected. Neutrophilia and lymphocytopenia also correlated with disease severity and mortality.[2,19]

To mount an antiviral response, the innate immune system recognizes molecular structures that are produced by the invasion of the virus, called pathogen-associated molecular patterns (PAMPs). For RNA virus such as coronavirus, it is known that PAMPs found in replicating viruses in the form of viral genomic ssRNA or double-stranded RNA that are recognized by either the endosomal RNA receptors, TLR8, and TLR7 in ssRNA and the cytosolic RNA sensor, RIG (retinoid-inducible gene)/MDA5 (melanoma differentiation-associated gene 5).[14,20,21] This recognition event leads to activation of several signaling pathways and ultimately transcription factors, that is, nuclear factor κB (NF-κB), activator protein 1 (AP-1), interferon response factor 3 (IRF3), and IRF7 accompanied by their nuclear translocation. NF-κB and AP-1 stimulate the expression of genes encoding many of the molecules required for inflammatory responses, including inflammatory cytokines (eg, tumor necrosis factor [TNF] and IL-1) and chemokines (eg, CCL2 and CXCL8). IRF3 and IRF7

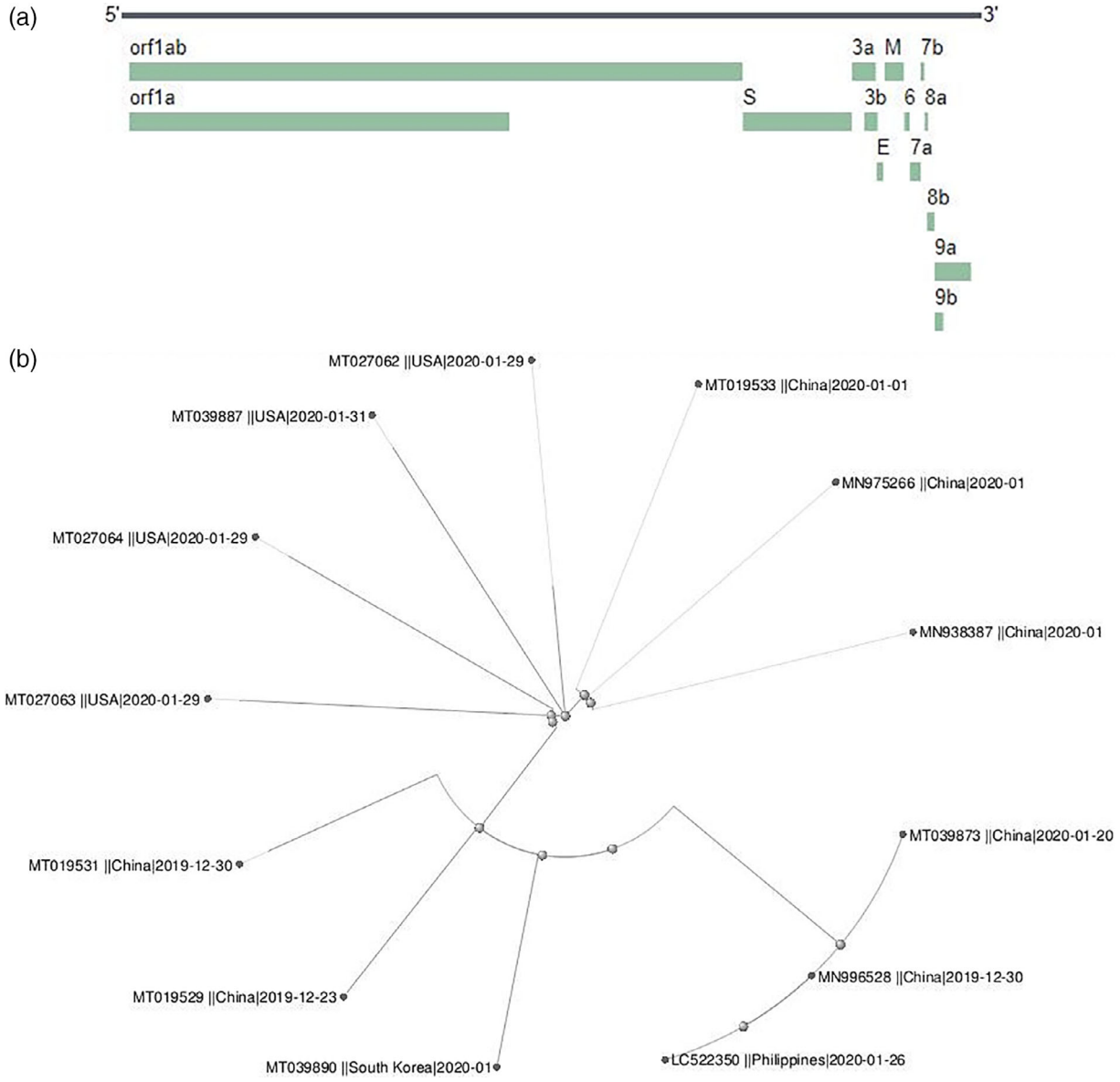

FIGURE 2 The genomic structure and phylogenetic tree of coronavirus SARS-CoV-2. A, The genome structure of the new coronavirus. B, The phylogenetic tree of representatives from different countries

promote the production of type I interferon (IFN-α and IFN-β), which are important for antiviral innate immune responses and able to suppress viral replication and dissemination at an early stage.[22,23] This process can cause complications such as exhaustion, weakness, and cough in patients.[24] The important point is, for SARS-CoV-2, the response to viral infection by type I IFN is suppressed (Figure 3).

5 | HUMORAL IMMUNITY TO SARS-COV-2 INFECTION

Humoral immune response, especially the production of neutralizing antibody, plays a protective role by limiting the infection at a later phase and prevents re-infection in the future.[26] SARS-CoV-2 infection induces IgG production against N protein that can be detected by serum as early as day 4 after the onset of disease and with most patients seroconverting by day 14.[27,28] Based on immunofluorescence assays and ELISA, in 89% of the recovered patients, IgG-specific and neutralizing antibodies were detected 2 years after SARS infection.[26,28,29] In addition, peak specific IgM on the ninth day after disease and the class switching to IgG in the second week were detected.[27,29,30]

During the long-term follow-up of survivors, IgG is only detectable in recovered patients at 6 years after SARS infection,[31,32] suggesting decreasing levels of memory B-cells will also be found against SARS-CoV-2.[31]

6 | T-CELL IMMUNITY TO SARS-COV-2

Laboratory evidence of clinical patients showed that specific T-cell responses against SARS-CoV-2 is important for the recognition and

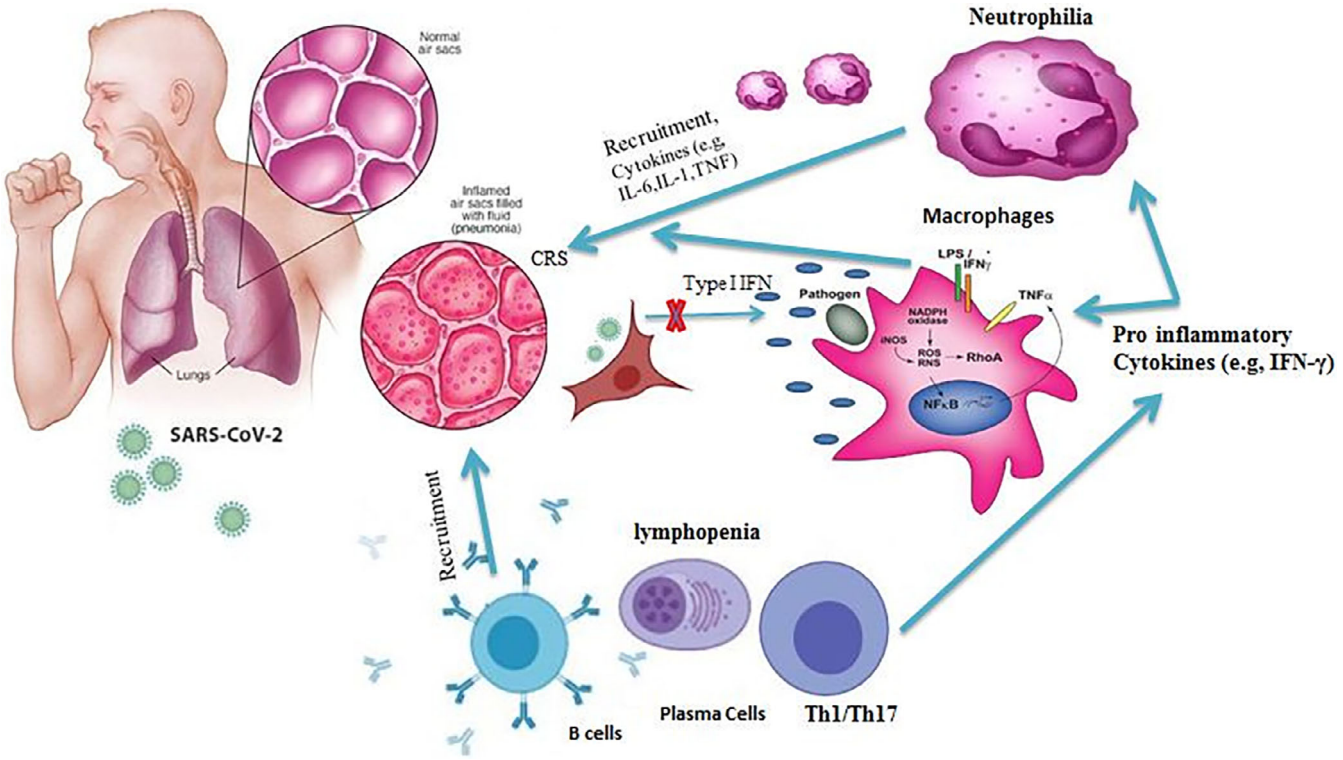

FIGURE 3 Host immune responses during SARS-CoV-2 infection. Airborne SARS-CoV-2 leads to infection of ACE2 expressing target cells such as alveolar type 2 cells or other unknown target cells. Cells infected by the virus may escape IFN I resulting in uncontrolled viral replication. The recruitment of neutrophils and monocytes/macrophages is by chemotaxis of pro-inflammatory cytokines. The "CRS" production-specific Th1/Th17 may cause immunopathologic injury in the lung that leads to pneumonia. B cells or plasma cells produce SARS-CoV-2 specific antibodies that may help neutralize viruses.[25] Lymphopenia caused by viral infections such as SARS-CoV-2 can occur with three mechanisms: The first mechanism is the reduction of lymphocyte production or impaired lymphopoiesis. The second mechanism is apoptosis and destruction of lymphocytes. The third mechanism that reduces lymphopenia without decreasing production or increasing degradation is lymphocyte redistribution, such as lymphocyte attachment to the vascular endothelium (a phenomenon similar to neutrophil marginalization) that can lead to decrease in circulating lymphocytes

killing of infected cells, particularly in the lungs of infected individuals.[33] The results of a study with 128 cases showed that the number and function of $CD8^+$ T cells were greater than $CD4^+$ T cell responses,[14,34] although whether the memory T-cell response is sufficient to protect from reinfection needs further study.[34-36] Furthermore, the virus-specific T cells from the severe infection tended to have a central memory phenotype with a significantly higher frequency of polyfunctional $CD4^+$ T cells with cytokine secretion, for example, IFNγ, TNFα, and IL-2, and $CD8^+$ T cells with cytokine secretion, for example, IFNγ, TNFα and degranulated state, as compared with mild infections. Strong T cell responses have a relationship with higher neutralizing antibody, while more serum Th2 cytokine secretion, for example, IL-4, IL-5, IL-10 (which increases the production of antibodies), were diagnosed in the deceased patients.[37-39] Contrary to the decrease in serum antibody levels in patients, cytotoxic T lymphocyte (CTL) function-specific N proteins are still detectable from the PBMCs of recovered patients from SARS or MERS more than 10 years post infection.[31,40-42]

7 | TREATMENT AND PREVENTION

At present, there is no single specific antiviral therapy for COVID-19 and the main treatments are supportive. Recombinant IFN with ribavirin only has limited effects against SARS-CoV-2 infection.[43] Glucocorticoids are not a routine treatment. In emergency cases, such as SpO2 < 90%, dexamethasone 5-10 mg or methylprednisolone 40-80 mg is given intravenously before transfer. High-throughput oxygen therapy and continuous positive airway pressure (CPAP) ventilation are both effective supportive therapies and target blood SpO2 should be 88%-90%. Invasive mechanical ventilation is used as a last resort.[44] The results of a recent study with 100 patients have demonstrated that chloroquine phosphate is more effective than control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus-negative conversion, and shortening the disease course.[45] In addition, teicoplanin, an antibiotic used to treat staphylococci infection, previously has been shown to be effective in inhibiting the first stage of MERS-coronavirus viral cycle in human cells. This activity is conserved on the SARS-CoV-2, thus placing teicoplanin as a potential treatment for patients with this virus.[46] Recently, tocilizumab, also known as atlizumab, prescribed for two patients with COVID-19 infection in Iran and has shown promising results. This drug is an immunosuppressive humanized monoclonal antibody against the interleukin-6 receptor (IL-6R) and mainly used for the treatment of rheumatoid arthritis (RA) and IL-6 is a cytokine that plays an important role in immune response.[18,47] More studies

with controls and large sample size are needed to evaluate the effect of this drug on the treatment of COVID-19 symptoms.

8 | CONCLUSION

We reviewed innate and acquired immune responses and the development of immune protection cells of patients who recovered from COVID-19, which could be a starting point for the design of prophylactic vaccines and immunotherapy-based therapy against coronaviruses. In addition, vaccines that stimulate both the cellular and humoral immune response against coronaviruses should also be considered.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

1. Wang LF, Shi Z, Zhang S, Field H, Daszak P, Eaton BT. Review of bats and SARS. *Emerg Infect Dis*. 2006;12(12):1834-1840.
2. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579(7798):265-269.
3. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature*. 2013;503(7477):535-538.
4. Zu ZY, Jiang MD, Xu PP, et al. Coronavirus disease 2019 (COVID-19): a perspective from China. *Radiology*. 2020;200490.
5. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. *Lancet*. 2020;395(10223):470-473.
6. Guo YR, Cao QD, Hong ZS, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. *Mil Med Res*. 2020;7(1):11.
7. Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. *J Med Virol*. 2020.
8. Shen Z, Xiao Y, Kang L, et al. Genomic diversity of SARS-CoV-2 in coronavirus disease 2019 patients. *Clin. Infect. Dis.* 2020.
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497-506.
10. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet*. 2020;395(10223):514-523.
11. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003;426(6965):450-454.
12. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. *Science*. 2020;367(6485):1444-1448.
13. Sun Z, Thilakavathy K, Kumar SS, He G, Liu SV. Potential factors influencing repeated SARS outbreaks in China. *Int. J. Environ. Res. Public Health*. 2020;17(5):1633.
14. Li G, Fan Y, Lai Y, et al. Coronavirus infections and immune responses. *J Med Virol*. 2020;92(4):424-432.
15. Baruah V, Bose S. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. *J Med Virol*. 2020;92(5):495-500.
16. Zhang B, Zhou X, Qiu Y, et al. *Clinical characteristics of 82 death cases with COVID-19*. medRxiv. 2020.
17. Wong CK, Lam CW, Wu AK, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol*. 2004;136(1):95-103.
18. Conti P, Ronconi G, Caraffa A, et al. Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by COVID-19: anti-inflammatory strategies. *J. Biol. Regul. Homeostatic Agents*. 2020;34(2).
19. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the clinical characteristics of coronavirus disease 2019 (COVID-19). *J Gen Intern Med*. 2020:1-5.
20. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. *Nat Rev Microbiol*. 2016;14(8):523-534.
21. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol*. 2017;39(5):529-539.
22. Deng X, van Geelen A, Buckley AC, et al. Coronavirus Endoribonuclease activity in porcine epidemic diarrhea virus suppresses type I and type III interferon responses. *J Virol*. 2019;93(8):e02000-18.
23. Yang CH, Li K, Pfeffer SR, Pfeffer LM. The type I IFN-induced miRNA, miR-21. *Pharmaceuticals*. 2015;8(4):836-847.
24. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS coronaviruses with the antiviral interferon response. *Adv Virus Res*. 2016;96:219-243.
25. Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. *Asian Pac J Allergy Immunol*. 2020;38:1-9.
26. Gorse GJ, Donovan MM, Patel GB. Antibodies to coronaviruses are higher in older compared with younger adults and binding antibodies are more sensitive than neutralizing antibodies in identifying coronavirus-associated illnesses. *J Med Virol*. 2020;92(5):512-517.
27. Liu W, Fontanet A, Zhang PH, et al. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. *J Infect Dis*. 2006;193(6):792-795.
28. Hsueh PR, Huang LM, Chen PJ, Kao CL, Yang PC. Chronological evolution of IgM, IgA, IgG and neutralisation antibodies after infection with SARS-associated coronavirus. *Clin Microbiol Infect*. 2004;10(12):1062-1066.
29. Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol*. 2020.
30. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. *J Virol*. 2007;81(16):8692-8706.
31. Tang F, Quan Y, Xin Z-T, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. *J Immunol*. 2011;186(12):7264-7268.
32. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273.
33. Gu J, Gong E, Zhang B, et al. Multiple organ infection and the pathogenesis of SARS. *J Exp Med*. 2005;202(3):415-424.
34. Channappanavar R, Fett C, Zhao J, Meyerholz DK, Perlman S. Virus-specific memory CD8 T cells provide substantial protection from lethal severe acute respiratory syndrome coronavirus infection. *J Virol*. 2014;88(19):11034-11044.

35. Chen J, Lau YF, Lamirande EW, et al. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. *J Virol*. 2010;84(3):1289-1301.
36. Zhao J, Zhao J, Perlman S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. *J Virol*. 2010;84(18):9318-9325.
37. Li CK, Wu H, Yan H, et al. T cell responses to whole SARS coronavirus in humans. *J Immunol*. 2008;181(8):5490-5500.
38. Fan YY, Huang ZT, Li L, et al. Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. *Arch Virol*. 2009;154(7):1093-1099.
39. Peng H, Yang LT, Wang LY, et al. Long-lived memory T lymphocyte responses against SARS coronavirus nucleocapsid protein in SARS-recovered patients. *Virology*. 2006;351(2):466-475.
40. Guan WD, Mok CK, Chen ZL, et al. Characteristics of traveler with Middle East respiratory syndrome, China, 2015. *Emerg Infect Dis*. 2015;21(12):2278-2280.
41. Ng OW, Chia A, Tan AT, et al. Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. *Vaccine*. 2016;34(17):2008-2014.
42. Oh HL, Chia A, Chang CX, et al. Engineering T cells specific for a dominant severe acute respiratory syndrome coronavirus CD8 T cell epitope. *J Virol*. 2011;85(20):10464-10471.
43. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr H. Treatment of SARS with human interferons. *Lancet*. 2003;362(9380):293-294.
44. Chen RC, Tang XP, Tan SY, et al. Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience. *Chest*. 2006;129(6):1441-1452.
45. Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends*. 2020;14(1):72-73.
46. Baron SA, Devaux C, Colson P, Raoult D, Rolain JM. Teicoplanin: an alternative drug for the treatment of coronavirus COVID-19? *Int J Antimicrob Agents*. 2020;105944.
47. Scott LJ. Tocilizumab: a review in rheumatoid arthritis. *Drugs*. 2017;77(17):1865-1879.

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