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Early Insights into Immune Responses during COVID-19

Ashley L. St. John^{*,†,‡,§} and Abhay P. S. Rathore[§]

Coronavirus disease-2019 (COVID-19) is caused by the newly emerged virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and was recently declared as a pandemic by the World Health Organization. In its severe form, the disease is characterized by acute respiratory distress syndrome, and there are no targeted intervention strategies to treat or prevent it. The immune response is thought to both contribute to the pathogenesis of disease and provide protection during its resolution. Thus, understanding the immune response to SARS-CoV-2 is of the utmost importance for developing and testing vaccines and therapeutics. In this review, we discuss the earliest knowledge and hypotheses of the mechanisms of immune pathology in the lung during acute infection as well as the later stages of disease resolution, recovery, and immune memory formation. *The Journal of Immunology*, 2020, 205: 000–000.

In December 2019, the city of Wuhan, China, the capital of Hubei province, began to experience a troubling increase in the incidence of viral pneumonia. This new disease was quickly identified to be caused by a novel coronavirus, SARS-CoV-2 (previously called 2019-nCoV), which likely first infected humans as the result of a zoonotic transmission event (1, 2). Similar to the recently emerged coronaviruses, severe acute respiratory syndrome (SARS)–CoV and Middle East respiratory syndrome (MERS)–CoV, severe disease caused by SARS-CoV-2 is characterized by acute respiratory distress syndrome (ARDS) (3, 4). These viruses are examples of betacoronaviruses, which are enveloped, positive-sense, ssRNA viruses (5). In fewer than four months following identification of the presumed initial infection cluster, the new disease, coronavirus disease-2019 (COVID-19), was designated as a pandemic by the World Health Organization and had resulted in unprecedented strain on medical institutions and disruptions to the economic and social networks of the world. We review our initial understanding of COVID-19 immunity and immune pathology through

building a description of the kinetics of immune responses from the early acute to resolution/recovery stages of infection in patients with mild and severe disease. We also discuss current hypotheses for the cellular and molecular basis of damage to the lung tissue and the implications of systemic immune responses for long-term immunity against SARS-CoV-2. Some of the data discussed are reported in preprints, so we caution that interpretations may evolve as those undergo peer review and as new studies are published in the field.

Signs, symptoms, and transmission

The initial presentation of COVID-19 is similar to other respiratory viral infections. Fever and cough are the most common signs at presentation, followed by muscle aches, confusion, and more rarely, sore throat, rhinorrhea, and chest pain (6, 7) (Fig. 1). The COVID-19 cough is often characterized as “dry,” and patients may have labored or rapid breathing (6, 8). There may also be disturbances to taste and smell perception (D. H. Brann, T. Tsukahara, C. Weinreb, M. Lipovsek, K. Van den Berge, B. Gong, R. Chance, I. C. Macaulay, H.-j. Chou, R. Fletcher, D. Das, K. Street, H. R. de Bezieux, Y.-G. Choi, D. Risso, S. Dudoit, E. Purdom, J. S. Mill, R. A. Hachem, H. Matsunami, D. W. Logan, B. J. Goldstein, M. S. Grubb, J. Ngai, and S. R. Datta, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.03.25.009084>) (Fig. 1). Virus is shed from the nasopharyngeal tract and can be detected in fecal specimens (9), although it has not been determined whether this reflects true gastrointestinal tract infection or if PCR-based detection results from gut permeability because infectious virus has not yet been isolated from the stool (10). The median shedding from the upper respiratory tract is ~10–12 days with persistent shedding up to 28 days in some patients, whereas detection in fecal samples persists ~20 days (W. Tan, Y. Lu, J. Zhang, J. Wang, Y. Dan, Z. Tan, X. He, C. Qian, Q. Sun, Q. Hu, H. Liu, S. Ye, X. Xiang, Y. Zhou, W. Zhang, Y. Guo, X.-H. Wang, W. He, X. Wan, F. Sun, Q. Wei, C. Chen, G. Pan, J. Xia, Q. Mao, Y. Chen, and G. Deng, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.24.20042382>; A. Wajnberg,

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Abbreviations used in this article: ACE2, angiotensin converting enzyme 2; ARDS, acute respiratory distress syndrome; BAL, bronchial alveolar lavage fluid; COVID-19, coronavirus disease-2019; MERS, Middle East respiratory syndrome; MIS-C, multisystem inflammatory disease in children; NHP, nonhuman primate; S, spike; SARS, severe acute respiratory syndrome.

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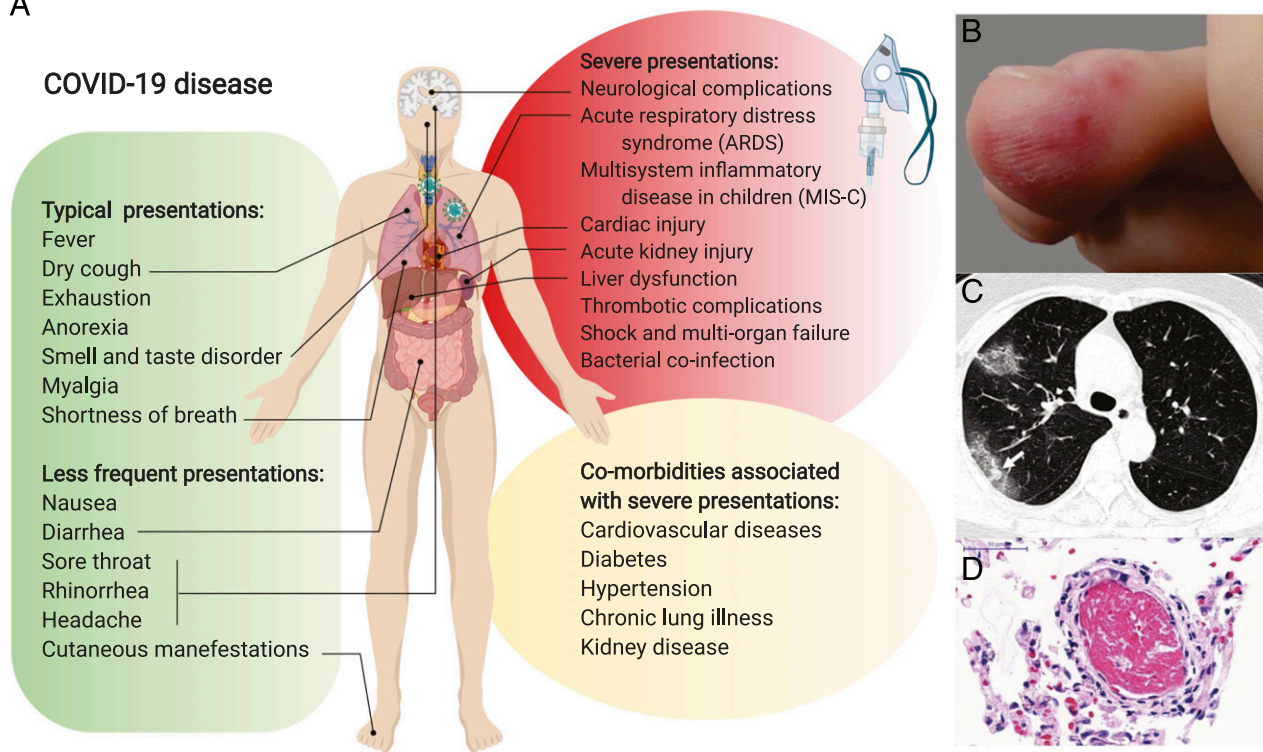


FIGURE 1. Clinical disease presentations of COVID-19. **(A)** The disease begins with signs and symptoms including fever, dry cough, shortness of breath, smell and taste disorder, and exhaustion (6, 94–96). Other less common presentations are sore throat, rhinorrhea, nausea, and diarrhea (7, 18). Some patients may also develop severe presentations, such as ARDS or, more rarely, cardiac, kidney and liver injuries (97), or vascular disease (31, 32). Although with lower incidence, bacterial coinfections, septic shock, and multiorgan failure are also reported (6, 18). Children can experience MIS-C (35). Several comorbidities have been identified as risk factors for severe clinical presentations. These include cardiovascular diseases, diabetes, hypertension, and chronic kidney and lung diseases (18). **(B)** An example of an erythematous lesion on the toe of a COVID-19 patient, reprinted from Landa, et al. (15). **(C)** Evidence of radiological finding of ground glass opacity in the lung of COVID-19 patient, seen by computed tomography (CT) scan 5 d after clinical presentation. Reprinted from Kong, et al. (98). © 2020 Radiological Society of North America. **(D)** Histological assessments reveal a microthrombus in the lung of a fatal COVID-19 case. Scale bar, 50 μ m. Reprinted from Dolhnikoff, et al. (21).

M. Mansour, E. Leven, N. M. Bouvier, G. Patel, A. Firpo, R. Mendu, J. Jhang, S. Arinsburg, M. Gitman, J. Houldsworth, I. Baine, V. Simon, J. Aberg, F. Krammer, D. Reich, and C. Cordon-Cardo, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.30.20085613> (Fig. 2). Another preliminary study indicates that transmissibility peaks around 14 h prior to symptom onset following an incubation period of ~5 days (Ref. 11 and W. Wu, Z. Xu, Y. Jin, and A. Pan, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.03.20030775>) (Fig. 2). SARS-CoV-2 is primarily transmitted by fomites and aerosols, particularly those generated by coughing (12–14). Increasingly, cutaneous manifestations, rashes, and erythematous lesions have been reported on the extremities, particularly in mild cases (15) (Fig. 1B). There are currently no approved targeted therapeutics against COVID-19, although many trials are ongoing and the antiviral drug remdesivir was given emergency use authorization in severe COVID-19 patients, based on early promising results in clinical trials (16, 17). Its further approval will depend on the results of ongoing safety and efficacy studies.

Although epidemiological data suggest that a substantial portion of patients experience mild or asymptomatic disease (18), hospitalized patients show bilateral or unilateral pneumonia, often with multiple mottling and ground-glass opacity (6), which are likely to be indicative of inflammation in the lung tissue (19) (Fig. 1C). Lesions in the lung of COVID-19

patients were larger than influenza patients when measured by chest computed tomography in one study (M. Zhou, Y. Chen, D. Wang, Y. Xu, W. Yao, J. Huang, X. Jin, Z. Pan, J. Tan, L. Wang, Y. Xia, L. Zou, X. Xu, J. Wei, M. Guan, J. Feng, H. Zhang, and J. Qu, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.24.20043117>). Lung lesions peaked the second week of clinical disease, and extensive lesions appear to correlate with poor prognosis (K. Li, D. Chen, S. Chen, Y. Feng, C. Chang, Z. Wang, N. Wang, and G. Zhen, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.23.20041673>). The earliest reports of infections in China suggested that 81% of cases are mild, 14% are severe, requiring supportive care, and 5% result in critical illness (18), although these rates are likely to vary depending on the clinical setting and population demographics. Onset of ARDS occurred an average of 10.5 days after onset of symptoms in one study (20).

Concurrent with signs of inflammation in the lungs, there is evidence of systemic modulation of the immune response. Coagulopathy is observed (21) (Fig. 1D), and lymphopenia is common (Fig. 1), reflecting reductions in circulating total CD45⁺ (hematopoietic lineage) cells, T cells, B cells, and NK cells (Y. Zheng, Z. Huang, G. Ying, X. Zhang, W. Ye, Z. Hu, C. Hu, H. Wei, Y. Zeng, Y. Chi, C. Cheng, F. Lin, H. Lu, L. Xiao, Y. Song, C. Wang, Y. Yi, and L. Dong, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.02.19.20024885>;

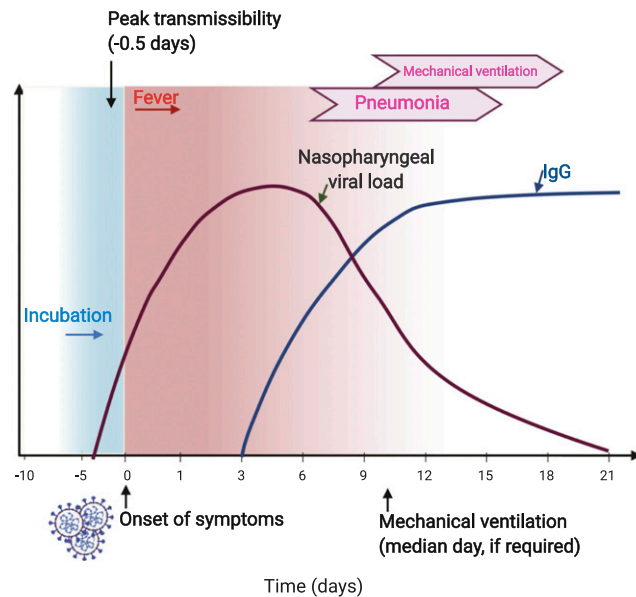


FIGURE 2. Time course of SARS-CoV-2 infection and illness in humans. After exposure to SARS-CoV-2, the median incubation period of virus is ~5 d (99). The peak transmissibility of virus is thought to be 0.5 d before symptoms arise (11). The onset of fever begins a clinical disease course which can last up to 3 wk (7). The median duration of fever is 4 d (0–14) with peak nasopharyngeal viral load detected between 3 and 5 d (7, 100). Concurrent with infection, nearly 50% of patients will seroconvert against the virus by day 5, with 100% seroconversion by day 14 (10). At this point, clinical symptoms will begin to resolve; however, some patients may experience severe disease characterized by ARDS and may require mechanical ventilation (6, 101). For those patients, the median duration of developing ARDS is 8 d after symptom onset (6).

A. J. Wilk, A. Rustagi, N. Q. Zhao, J. Roque, G. J. Martinez-Colon, J. L. McKechnie, G. T. Ivison, T. Ranganath, R. Vergara, T. Hollis, L. J. Simpson, P. Grant, A. Subramanian, A. J. Rogers, and C. A. Blish, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.17.20069930>, although there may also be elevated leukocytes during early disease at the time of admission (6). In surviving patients, lymphocyte levels were higher and recovered faster than those of non-survivors (22, 23), and faster lymphocyte recovery has also been associated with virus control (Refs. 24, 25, and X. Chen, J. Ling, P. Mo, Y. Zhang, Q. Jiang, Z. Ma, Q. Cao, W. Hu, S. Zou, L. Chen, L. Yao, M. Luo, T. Chen, L. Deng, K. Liang, S. Song, R. Yang, R. Zheng, S. Gao, X. Gui, H. Ke, W. Hou, Å. Lundkvist, and Y. Xiong, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.03.20030437>), which support the notion that productive immune function is key to recovery from COVID-19. The mortality rate is highest in the middle aged and elderly, especially those with pre-existing conditions (18) (Fig. 1). In some reports, bacterial coinfections were present, requiring antibiotic treatment (6, 20, 26, 27), which may be consistent with the observation of elevated neutrophils in some studies (Refs. 6, 28, and J. Gong, H. Dong, S. Q. Xia, Y. Z. Huang, D. Wang, Y. Zhao, W. Liu, S. Tu, M. Zhang, Q. Wang, and F. Lu, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.02.25.20025643>). Another study identified a higher neutrophil to lymphocyte ratio as being associated with severe disease (29). For a portion of patients with severe disease, prolonged hypoxemia, or lowered oxygen in the arterial blood, occurs, and

mechanical ventilation is required, with a mean duration of 9 days of therapy (7, 20, 22). Mortality results from severe respiratory failure associated with ARDS but may also be due to or exacerbated by bacterial sepsis, heart failure, or multiorgan failure (6) (Fig. 1). Other organs with possible involvement include the kidney, liver, and gut, as evidenced by their apparent injury in some patients with severe disease (Refs. 22, 28, and J.-K. Sun, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.25.20043570>). One biomarker of acute tissue injury to the heart, high-sensitivity cardiac troponin I, was found to be elevated in patients with severe disease and associated with poor prognosis (30). Infrequent severe presentations include neurologic complications, including encephalitis, and thrombotic complications resulting in stroke (31–33) (Fig. 1A).

Although early reports from China suggested that children only rarely experienced severe disease (34), as COVID-19 spread into Europe and the Americas it became clear that a small minority of children develop severe respiratory disease and some also develop a hyperinflammatory syndrome that is clinically similar to Kawasaki disease (35, 36). Multisystem inflammatory disease in children (MIS-C) can manifest in the weeks following asymptomatic or mild infections and is associated with inflammatory disease affecting multiple organs, coagulopathy, and high rates of shock (35). MIS-C is associated with elevated serum C-reactive protein (CRP), ferritin, and *d*-dimers (35). At this time, the mechanisms of its pathophysiology are not known, but MIS-C emphasizes the broad range of disease outcomes that are associated with COVID-19 and the importance of studying disease in multiple patient populations, including pediatric patients.

Proposed mechanisms of immune pathology in the lung

At a cellular level, SARS-CoV-2 infects epithelial cells lining the airways (Fig. 3). Like other coronaviruses, including SARS-CoV, infection of these cells by SARS-CoV-2 is dependent on their expression of angiotensin converting enzyme 2 (ACE2), which binds to the viral envelope-associated spike (S) protein as a cellular receptor (37, 38) after proteolytic cleavage of both S and ACE2 by serine proteases (39). For this reason, mice with the human *ACE2* allele knocked-in are promising models for vaccine and drug testing (40). However, other promising models include nonhuman primates (NHPs), which appear to recapitulate the histological and radiological findings associated with human lung inflammation (Ref. 41 and C. Woolsey, V. Borisevich, A. N. Prasad, K. N. Agans, D. J. Deer, N. S. Dobias, J. C. Heymann, S. L. Foster, C. B. Levine, L. Medina, K. Melody, J. B. Geisbert, K. A. Fenton, T. W. Geisbert, and R. W. Cross, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.05.17.100289>; and C. L. Finch, I. Crozier, J. H. Lee, R. Byrum, T. K. Cooper, J. Liang, K. Sharer, J. Solomon, P. J. Sayre, G. Kocher, C. Bartos, N. M. Aiosa, M. Castro, P. A. Larson, R. Adams, B. Beitzel, N. Di Paola, J. R. Kugelman, J. R. Kurtz, T. Burdette, M. C. Nason, I. M. Feuerstein, G. Palacios, M. C. S. Claire, M. G. Lackemeyer, R. F. Johnson, K. M. Braun, M. D. Ramuta, J. Wada, C. S. Schmaljohn, T. C. Friedrich, D. H. O'Connor, and J. H. Kuhn, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.05.14.096727>), and ferrets and hamsters, which are both able to transmit infection in experimental settings (42, 43). In humans, ACE2 is highly expressed on the epithelial cells lining the bronchial alveoli (44),

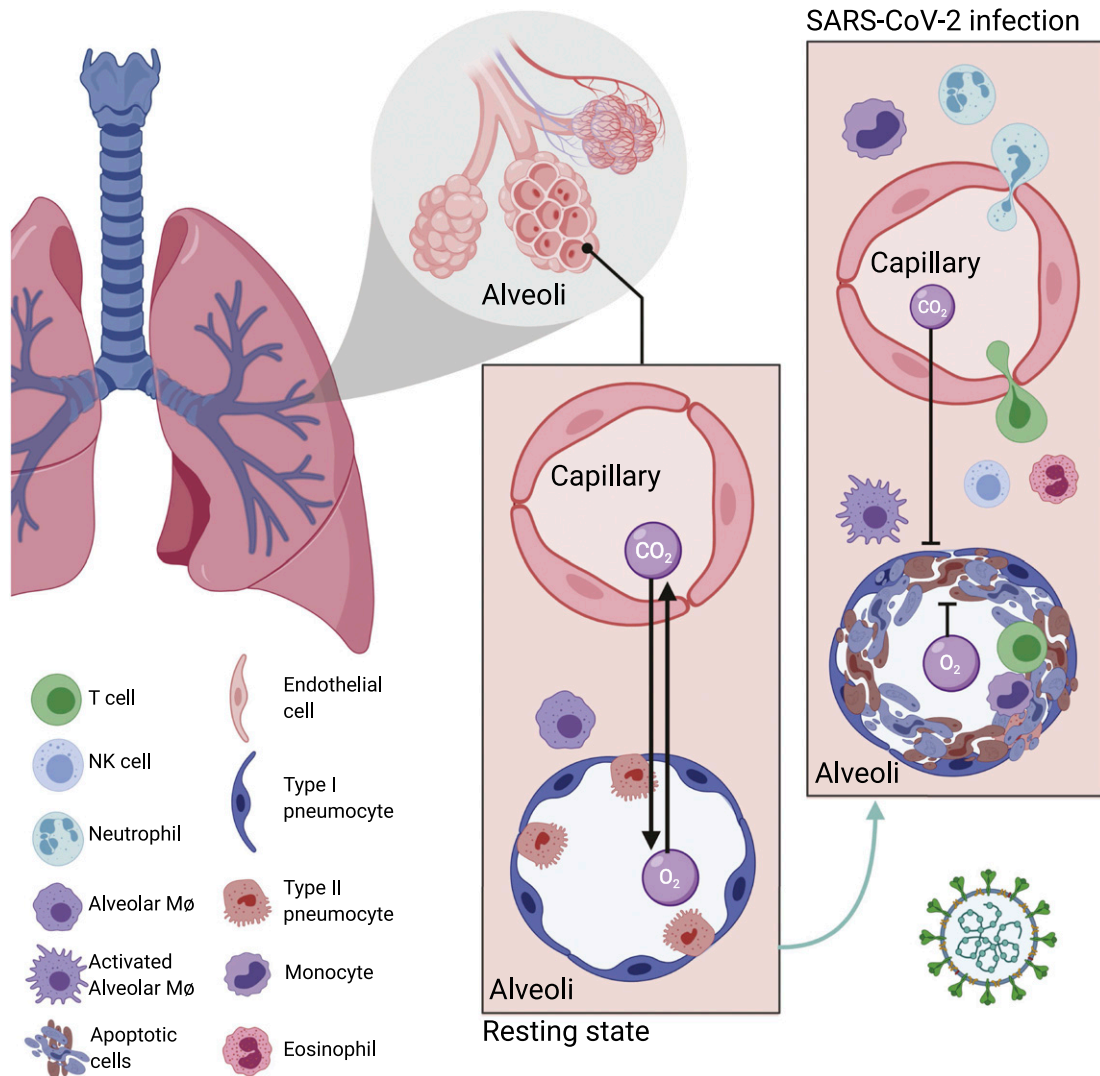


FIGURE 3. Immune pathology in SARS-CoV-2-infected lung tissue. The lung, the critical organ for gas exchange, is the target of SARS-CoV-2 infection. In healthy tissue, the alveolae at the ends of terminal bronchi are critical for the flow of oxygen, and few immune cells, such as alveolar macrophages, are located in the tissue but in a resting state. The proximity of capillaries to the alveoli allows the exchange of O_2 and CO_2 to replenish the O_2 supply in the blood. After SARS-CoV-2 infection, inflammation is induced in the tissue, involving cellular recruitment of many types of immune cells, including T cells, NK cells, neutrophils, inflammatory monocytes, and potentially others (28, 49). These immune cells have an activated phenotype and can even be found in the BAL (49). Tissue damage results in a hyaloid membrane, a layer of dead and dying cells in the alveoli that may limit gas exchange (48).

presumably allowing wide-spread infection at the lung–air interface, and is an IFN-stimulated gene, which could conceivably complicate the role of IFN in antiviral protection (45). Endothelial cells may also be infected, and in support, virus particles were observed within endothelial cells of the kidney (46). As infected cells are cleared by apoptosis or actions of the immune system (i.e., cytotoxic T cells), damage to the lung tissue there can lead to rapid turn-over of the epithelium. In lung tissue sections following autopsy of humans that died of either SARS (47) or COVID-19 (28, 48)–induced ARDS, a hyaline membrane, or layer of dead cells and debris, can be observed (Fig. 3). Histologic sections of lung tissue also revealed extensive infiltration of mononuclear cells (48), many of which are presumably monocytes and T cells, and the presence of thrombi in the pulmonary arterioles (48). Edema in the lung tissue is also observed (Ref. 48 and S. E. Fox, A. Akmatbekov, J. L. Harbert, G. Li, J. Q. Brown, and R. S. Vander Heide, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.06.20050575>). In a small study comparing transcriptional

signatures on cells of the bronchial alveolar lavage fluid (BAL) of patients with mild and severe disease, single-cell sequencing revealed reduced FABP4⁺ alveolar macrophages and increased FCN1⁺ inflammatory and SPP1⁺ profibrotic macrophages in patients with severe disease (49). Like the peripheral blood, lymphocytes, including NK and T cells, were reduced in severe compared with mild COVID-19 cases (49). The phenotypes of T cells from the BAL also differed with severity, with mild cases being characterized by a strong T cell activation profile of clonally expanded cells, whereas severe cases were characterized by metabolic activation and stress response pathways (49). Animal models also appear to recapitulate some aspects of the human presentation of disease, with experimentally infected rhesus macaques showing pneumonia on day 3 of infection that is characterized by alveolar edema, occasional bronchiole necrosis, fibrin deposition, formation of a hyaline membrane, and cellular infiltration, including infiltration of monocytes and neutrophils (41). Very similar immune pathologies were also observed day 4 postinfection in cynomolgus macaques (50).

Presumably these inflammatory events, combined with massive cell death of infected cells, contribute to ARDS by reducing the elasticity of the lung tissue and obstructing oxygen penetration (Fig. 3).

As studies in the lung tissue of humans or animal models infected by SARS-CoV-2 are limited, much of our information regarding the inflammatory profile *in vivo* is derived from detection of biomarkers in the serum or plasma of patients, which has a limitation of potentially not fully capturing the immune profile in the lung tissue. Severe COVID-19 patients who required intensive care unit admission were shown to have higher plasma levels of IL-2, IL-7, IL-10, G-CSF, IP-10, MIP1 α , and TNF (20, 51). Altered coagulation is also associated with severe disease, including elevated *d*-dimers (6, 22, 51) and the presence of high levels of IL-6 (22, 51), which is now being considered as a possible therapeutic target, as is IL-1 (23). The association of elevated cytokines with severe disease in multiple studies (Refs. 20, 22, 29, 51–53, and J. Gong, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.02.25.20025643>) has led to the suggestion that cytokine storm maybe an underlying cause of disease and death, although further studies are needed to validate this. Although the efficacy of IL-6 blockade in preventing ARDS is not yet known, early data suggesting that it may be associated with increased secondary infections in COVID-19 patients supports the importance of proceeding with caution when suppressing immune responses (L. M. Kimmig, D. Wu, M. Gold, N. N. Pettit, D. Pitrak, J. Mueller, A. N. Husain, E. A. Mutlu, and G. M. Mutlu, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.05.15.20103531>). In contrast to the assumption that a robust inflammatory response is characteristic of disease, transcriptional analysis of human lung epithelial cells showed a muted proinflammatory response characterized by decreased type I and type III IFN expression compared with other respiratory viral pathogens, such as respiratory syncytial virus and influenza A, despite effective induction of many innate immune signaling intermediates and activation of cytosolic sensors of viral RNA, including RIG-I and OAS1-3 (54). Another report indicates that SARS-CoV-2 is a very weak inducer of type I IFNs, IFN- α/β , in human lung tissue compared with SARS-CoV (55). Similarly, in a ferret model of infection, levels of key cytokines were lower in the tracheas of SARS-CoV-2-infected animals than influenza-infected animals at day 5 postinfection, although detection of virus genome copies suggested adequate infection levels in the model (54). Although 5 days postinfection represents a single time point and the full kinetics of the inflammatory response remain to be described in animal models, these results caution that more information is needed before assuming that cytokine storm alone is the cause of severe disease. Consistent with a muted cytokine response, cytokine levels were only significantly elevated in the serum of SARS-CoV-2-infected rhesus macaques 1 day postinfection, resolving thereafter, whereas pulmonary infiltration continued to progress to day 3 and was apparent by histology (41). In these rhesus macaques, lesions in the lungs remained to day 21 postinfection in 50% of animals assessed at that time point (41).

There are also inflammatory pathways that are cytokine independent, which could contribute to disease. For example, preliminary results suggest that complement activation could contribute to lung pathology, potentially through complement

C4 cleavage, which may be modulated by the lectin MASP-2 pathway activation by the SARS-CoV-2 N protein. This pathway also appears to be activated by N protein from SARS and MERS (T. Gao, M. Hu, X. Zhang, H. Li, L. Zhu, H. Liu, Q. Dong, Z. Zhang, Z. Wang, Y. Hu, Y. Fu, Y. Jin, K. Li, S. Zhao, Y. Xiao, S. Luo, L. Li, L. Zhao, J. Liu, H. Zhao, Y. Liu, W. Yang, J. Peng, X. Chen, P. Li, Y. Liu, Y. Xie, J. Song, L. Zhang, Q. Ma, X. Bian, W. Chen, X. Liu, Q. Mao, and C. Cao, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.29.20041962>). Complement split products, including anaphylatoxins C3a and C5a, are known to induce inflammation in the lung tissue when present and may activate local lung-resident immune cells, such as mast cells, and promote the recruitment of other immune cell types, including basophils and eosinophils (56–58). Although additional studies are needed to confirm the role of complement in animal models, a correlation between the levels of C5a and severity of COVID-19 disease has been suggested (T. Gao, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.29.20041962>).

Hallmarks of systemic COVID-19 immunity and disease

Altered coagulation maybe a major factor in COVID-19 severity, and extensive intravascular coagulation was shown to be associated with poor disease prognosis (59). This is also consistent with the elevated *d*-dimer detection in patient blood during severe cases because *d*-dimer is a degradation product of cross-linked fibrin and indicates the presence of fibrin deposition (60). Indeed, in a recent study involving autopsies from fatal COVID-19 cases, extensive bilateral pulmonary edema with hemorrhage was noted, and thrombi were present in blood vessels (S. E. Fox, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.06.20050575>).

SARS-CoV-2 also infects secondary lymphoid organs, which has been postulated to be the cause of lymphopenia that is associated with severe disease because this coincides with atrophy of the white pulp of the spleen (Y. Chen, Z. Feng, B. Diao, R. Wang, G. Wang, C. Wang, Y. Tan, L. Liu, C. Wang, Y. Liu, Y. Liu, Z. Yuan, L. Ren, and Y. Wu, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.27.20045427>). Viral Ag has been detected in CD68⁺ and CD169⁺ macrophages in the white pulp of the human spleen and within lymph node sinuses, presumably using macrophage expression of the ACE2 to infect these cells (Y. Chen, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.27.20045427>). Within the spleen, apoptotic lymphocytes and areas of necrosis were also observed during human postmortem examination (Y. Chen, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.27.20045427>).

Risk factors for severe disease in the context of lung homeostasis

Low oxygen at presentation was associated with more severe disease and poor clinical outcomes (61), likely reflecting the importance of the lung for gas exchange and the potential of infection and inflammation to impair this essential physiological process (Fig. 3). Other risk factors for severe disease or mortality identified thus far include hypertension, smoking, coronary heart disease, cerebrovascular disease, diabetes, and pre-existing lung diseases such as chronic obstructive pulmonary

disease (8, 20, 62) (Fig. 1). Chronic infections, including hepatitis B virus or tuberculosis, may also be associated with severe disease (X. Chen, Q. Jiang, Z. Ma, J. Ling, W. Hu, Q. Cao, P. Mo, R. Yang, S. Gao, X. Gui, Y. Xiong, J. Li, and Y. Zhang, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.23.20040733>; Y. Liu, L. Bi, Y. Chen, Y. Wang, J. Fleming, Y. Yu, Y. Gu, C. Liu, L. Fan, X. Wang, and M. Cheng, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.10.20033795>). Many of these comorbidities and pre-existing conditions are characterized by underlying changes to inflammation and immune homeostasis, and it will be important to identify immune factors that contribute to increased susceptibility for severe disease in these patient populations. From SARS, we can see that host genetics influencing immunity can be a factor because, for example, certain human HLAs are associated with more severe disease (63). Early bioinformatics analysis suggests that this same HLA, HLA-B*46:01, may also be a susceptibility marker for COVID-19 disease because it is predicted to present very few viral peptides compared with other similar MHC class I molecules (64), which could conceivably influence the ability of CD8 T cells to recognize infected cells.

Multiple studies indicate that men are more susceptible to severe disease and death than women (65–67), although it is not yet understood whether this is due to an underlying sex-based immunological or physiological mechanism or other factors. Surprisingly, however, COVID-19 infection has not been associated with more severe outcomes in pregnant women compared with the general population, in contrast to the diseases caused by closely related viruses SARS-CoV and MERS-CoV (R. Nie, S.-s. Wang, Q. Yang, C.-f. Fan, Y.-l. Liu, W.-c. He, M. Jiang, C.-c. Liu, W.-j. Zeng, J.-l. Wu, K. Oktay, L. Feng, and L. Jin, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.22.20041061>). Perinatal transmission was also rare (R. Nie, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.22.20041061>), although infection was associated with preterm labor and the need for cesarean section in one study (68).

Resolution, recovery, and immune memory

Recovery from COVID-19 may also be associated with persistent immunological changes. Some patients continue to have reduced numbers of lymphocytes and eosinophils in the weeks after hospital discharge (Refs. 24, 69, and F. Zhou, X. Yu, X. Tong, and R. Zhang, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.26.20041426>) as well as altered renal function and coagulation, as shown by increased blood urea nitrogen, creatinine, fibrinogen, and *d*-dimers (F. Zhou, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.26.20041426>), although other studies showed that lymphocytes returned to normal (X. Xue, J. Ma, Y. Zhao, A. Zhao, X. Liu, W. Guo, F. Yan, Z. Wang, Y. Guo, and M. Fan, manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.27.20040816>). Interestingly, single-cell sequencing has identified that CD4 and CD8 T cells show a large degree of clonal expansion and conversion to memory phenotypes, despite their reduced numbers in the blood (69). ELISPOT analysis also reveals the functionality of T cells, which are able to secrete IFN- γ in response to ex vivo stimulation with SARS-CoV-2 proteins, ~2 weeks following discharge from the hospital (70). During recovery, other cell types

in the blood were increased, including proinflammatory CD14⁺ monocytes and NK cells (69). Despite the overall reduction in B cell counts in the blood, this reduction was largely attributable to reduced circulating naive B cells, and there is no indication of defects in plasma cell production because these cells are also expanded in the weeks following infection (F. Zhou, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.26.20041426>). Oddly, a novel B cell population may be present in the peripheral blood of patients with severe disease, characterized by class-switched B cells that also express proteins most commonly associated with granulocytes (A. J. Wilk, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.17.20069930>), but this remains to be confirmed in additional studies. Although it is too early to know how long Ag-specific T cell responses will persist to SARS-CoV-2, T cell responses to SARS-CoV were durable in survivors for over a decade (71).

Biomarkers of inflammation also persist at elevated levels after recovery, including of IL-6 and CRP (Y. Zheng, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.02.19.20024885>; F. Zhou, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.26.20041426>). As expected, following transient IgM detection early in infection, seroconversion is shown by detection of SARS-CoV-2-specific IgG in the serum (24). In one study of patients with mild disease, 50% of patients had seroconverted by day 7 postinfection, and 100% had seroconverted by day 14 (10), suggestive of a robust Ab response. SARS-CoV-2-specific IgA can also be detected in the serum (72), supporting induction of a virus-specific mucosal immune response in humans. Surprisingly, patients with severe disease have been shown to have higher IgG Ab titers than patients with nonsevere disease (Ref. 29 and W. Tan, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.03.24.20042382>); however, it is not clear whether this correlation indicates a role for Abs in disease severity or whether severe disease is more effective in generating long-term immune responses that could still be protective. A serosurvey showed that >99% of confirmed COVID-19 patients effectively seroconvert (A. Wajnberg, et al., manuscript posted on medRxiv, DOI: <https://doi.org/10.1101/2020.04.30.20085613>). Most Abs identified in convalescent sera against the nucleocapsid (NP) and S proteins and were predominantly IgG1 (70), interestingly, a Th2-associated isotype (73, 74). Although nearly uniformly detectable following infection for SARS-CoV-2, studies of patients who recovered from SARS-CoV have raised questions regarding how long these Abs can persist because only 50% of patients were still seropositive 4 years postinfection (75). Low virus-specific Ab titers are reported in those who recovered from mild COVID-19 disease, although the humoral response was universally found to contain neutralizing Abs against the S protein receptor-binding domain (Ref. 70 and D. F. Robbiani, C. Gaebler, F. Muecksch, J. C. C. Lorenzi, Z. Wang, A. Cho, M. Agudelo, C. O. Barnes, S. Finkin, T. Hagglof, T. Y. Oliveira, C. Viant, A. Hurley, K. G. Millard, R. G. Kost, M. Cipolla, A. Gazumyan, K. Gordon, F. Bianchini, S. T. Chen, V. Ramos, R. Patel, J. Dizon, I. Shimeliovich, P. Mendoza, H. Hartweger, L. Nogueira, M. Pack, J. Horowitz, F. Schmidt, Y. Weisblum, H.-H. Hoffmann, E. Michailidis, A. W. Ashbrook, E. Waltari, J. E. Pak, K. E. Huey-Tubman, N. Koranda, P. R. Hoffman, A. P. West, C. M. Rice, T. Hatziioannou, P. J. Bjorkman, P. D. Bieniasz, M. Caskey,

and M. C. Nussenzweig, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.05.13.092619>). Neutralizing Ab titers were shown to be correlated with virus-specific T cells (70). Interestingly, Ab responses to MERS-CoV were also widely detected in camel workers in Saudi Arabia, potentially supporting the association of Abs with exposure to highly pathogenic coronaviruses in survivors (76). Although it is too early to understand how durable human immunity will be to SARS-CoV-2, that Ab and B cell responses appear to have appropriate magnitude and kinetics and that T cell responses are clonally expanded and converted to memory phenotypes is supportive that immunological memory should be lasting and protective for healthy individuals.

A serosurvey of a small number of healthy donors who provided samples prior to the emergence of SARS-CoV-2 indicated that pre-existing Abs to common human coronaviruses show very little cross-reactivity to SARS-CoV-2 (S. Khan, R. Nakajima, A. Jain, R. R. de Assis, A. Jasinskas, J. M. Obiero, O. Adenaiye, S. Tai, F. Hong, D. K. Milton, H. Davies, and P. L. Felgner, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.03.24.006544>). One preliminary study of a small cohort of SARS-CoV-2 immune individuals showed that there was no cross-reactivity of their Abs against the receptor-binding domain of S protein from SARS-CoV or MERS-CoV, and no cross-neutralization of the viruses (B. Ju, Q. Zhang, X. Ge, R. Wang, J. Yu, S. Shan, B. Zhou, S. Song, X. Tang, J. Yu, J. Ge, J. Lan, J. Yuan, H. Wang, J. Zhao, S. Zhang, Y. Wang, X. Shi, L. Liu, X. Wang, Z. Zhang, and L. Zhang, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.03.21.990770>). Most cross-reactive Abs are directed at the S2 domain of S protein and the NP protein (S. Khan, et al., manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.03.24.006544>), which is also a prime target of the subunit vaccines that are in development (77). Although limited, the presence of coronavirus cross-reactive immune responses raises the question whether aspects of immune interference, including original antigenic sin or Ab-dependent enhancement of infection [as is seen with flaviviruses (78) and the feline coronavirus that causes feline infectious peritonitis (79, 80)] could influence the pathogenesis of disease and possibly explain age-related differences in susceptibility to severe disease to a certain extent. This will be an important consideration during vaccine development to ensure that any vaccine advanced to humans has a good safety profile for exposure to SARS-CoV-2 as well as other seasonal coronaviruses. The influence of pre-existing Abs on disease severity is also important to understand to establish the safety of potential therapeutics such as convalescent plasma therapy or SARS-CoV-2-specific mAbs (81–83). Although there are some indications that convalescent plasma therapy maybe effective (81), a randomized placebo-controlled trial has not yet been performed, and the first described candidate mAbs have yet to be tested in vivo (82, 83).

Another consideration for vaccine development is the role of T cells in protection against SARS-CoV-2. For closely related viruses, SARS-CoV and MERS-CoV, a mucosal immunization protocol that induced high levels of memory CD4 T cells in the airways was shown to be critical for protection during rechallenge, as was induction of CD8 T cell responses (84). The protective airway-associated CD4 T cells were also multifunctional, as shown by the production of multiple cytokines and higher cytokine levels (84). Similarly, for

SARS-CoV, mucosal vaccination protocols have established that high levels of IgA are correlated with reduced viral loads and improved lung pathology (85, 86). These studies emphasize the importance of mucosal immunity for vaccine-induced protection from coronaviruses.

Several SARS-CoV-2 vaccine candidates are currently being developed at unparalleled speed, but the majority are in the initial stages of testing (87–89). Subunit vaccines, particularly those using the S protein and DNA- or RNA-based platforms are generally safe to administer, and their production can be ramped up quickly (90), but these still require testing for safety during a virus challenge in animal models and testing in humans. Recent preclinical studies provide an early glimpse of immune protection against SARS-CoV-2 through vaccination. For example, adenovirus-vectored vaccine ChAdOx1 nCoV-19, which encodes SARS-CoV-2 S protein, evoked a protective Th1-associated immune response in mice and rhesus macaques (N. van Doremalen, T. Lambe, A. Spencer, S. Belij-Rammerstorfer, J. N. Purushotham, J. R. Port, V. Avanzato, T. Bushmaker, A. Flaxman, M. Ulaszewska, F. Feldmann, E. R. Allen, H. Sharpe, J. Schulz, M. Holbrook, A. Okumura, K. Meade-White, L. Pérez-Pérez, C. Bissett, C. Gilbride, B. N. Williamson, R. Rosenke, D. Long, A. Ishwarbhai, R. Kailath, L. Rose, S. Morris, C. Powers, J. Lovaglio, P. W. Hanley, D. Scott, G. Saturday, E. de Wit, S. C. Gilbert, and V. J. Munster, manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.05.13.093195>). Vaccination reduced virus load in the BAL and respiratory tract and protected against pneumonia, although neutralizing Ab titers were low (N. van Doremalen, et al., manuscript posted on bioRxiv, DOI: <https://doi.org/10.1101/2020.05.13.093195>). In another study, a DNA vaccine encoding the full-length S protein evoked neutralizing Ab titers similar to the levels detected in convalescent human sera, activated CD4 and CD8 T cells, and provided protection during SARS-CoV-2 challenge (91). Formulations of inactivated SARS-CoV-2 virus are also being tested (88), and one such vaccine candidate (PiCoVacc) evoked neutralizing Abs in mice, rats, and NHPs (92). Importantly, neutralization of multiple SARS-CoV-2 strains was tested, and PiCoVacc also protected against SARS-CoV-2 challenge in NHPs (92), a reassuring development because immunization with inactivated cat coronaviruses has been associated with worsened disease through immune enhancement (93). The next phase for these vaccines and the many others in the pipeline will be to undertake safety and efficacy studies in humans. Longer-term goals of vaccination should include the development of vaccines to protect the most at-risk groups, such as the elderly, who are also often refractory to vaccines, and to cross-protect against coronaviruses to guard against the threat of future emergence of novel strains.

Conclusions

In acute viral respiratory infections, immune responses are believed to be essential for viral infection clearance and immunological memory, although they also cause collateral damage to the lung tissue that can be detrimental and even fatal in some cases. Understanding the phenotype and specificity of immunity to SARS-CoV-2 will be essential for developing a vaccine that is capable of protecting from infection without inducing immune pathology.

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