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Review Article

REVIEW ARTICLE

The Immunopathobiology of SARS-CoV-2 Infection

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One sentence summary: Severe acute respiratory syndrome causing coronavirus 2 (SARS-CoV-2) has set off a pandemic with more than 3.8 million COVID-19 casualties. Although several emergency authorized vaccines are effective against the circulating strains, it is more important than ever to provide comprehensive information on the immunopathology of this agent.

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ABSTRACT

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can lead to coronavirus disease 2019 (COVID-19). Virus-specific immunity controls infection, transmission and disease severity. With respect to disease severity, a spectrum of clinical outcomes occur associated with age, genetics, comorbidities and immune responses in an infected person. Dysfunctions in innate and adaptive immunity commonly follow viral infection. These are heralded by altered

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innate mononuclear phagocyte differentiation, activation, intracellular killing and adaptive memory, effector, and regulatory T cell responses. All of such affect viral clearance and the progression of end-organ disease. Failures to produce effective controlled antiviral immunity leads to life-threatening end-organ disease that is typified by the acute respiratory distress syndrome. The most effective means to contain SARS-CoV-2 infection is by vaccination. While an arsenal of immunomodulators were developed for control of viral infection and subsequent COVID-19 disease, further research is required to enable therapeutic implementation.

Keywords: SARS-CoV-2; ACE2; COVID-19 vaccines; cytokine storm; immunity; mutant viral variants

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the greatest global health threat of this century (Chen *et al*. 2020; Hui *et al*. 2020; Wu *et al*. 2020; Zhou *et al*. 2020). The evidence for this claim is strong. Of the three novel coronaviruses reported, SARS-CoV-2 has the deadliest virus-associated morbidities and mortalities. SARS-CoV-2 infections has eclipsed the 2012 Middle East Respiratory Syndrome coronavirus (MERS-CoV) and the 2003 SARS-CoV in human disease impact (da Costa, Moreli and Saivish 2020). Due to the number of SARS-CoV-2 infections and deaths reported, the World Health Organization (WHO) classified COVID-19 as the "highest level" global health threat (Zhong 2020). Apropos of the virus' clinical course, SARS-CoV-2 elicits its pathology by infecting lung epithelial cells. This resulted in innate immune activation, inflammation and respiratory impairment (Zhang *et al*. 2020). While this pathway results in antiviral immunity enabling viral clearance, it engages pathways linked to disease progression by affecting the brain, heart, kidney and liver. Organ failures, when they occur, are associated with dysfunctional innate and adaptive immune responses and limitations in viral clearance.

Thus far, the knowledge gained from SARS-CoV-2 infection and COVID-19 disease has propelled viral genome studies, therapeutics, social programs and the development and deployments of vaccines (Machhi *et al*. 2020). SARS-CoV-2 is a positive-sense single-stranded mRNA (+ssRNA) animal virus belonging to the family *Coronaviridae*, genus *Betacoronavirus*, and subgenus *Sarbecovirus*. Genetic and immunologic coronaviral similarities include an enveloped outer structure composed of crown-shaped spike proteins (Yu *et al*. 2020). Knowledge of the viral proteins and their function(s), pathways for its entry into cells, and its replication cycle have been determined. However, the means to suppress infection and alter the disease through therapeutic developments have been limited in their clinical effectiveness. Indeed, there remains tepid enthusiasm based on controlled clinical studies for the use of hydroxychloroquine, remdesivir, favipiravir and arbidol. These are all repurposed small-molecule drugs that possess varied antiviral activities in laboratory settings and in an infected human host (Liu *et al*. 2020;Zhou *et al*. 2020). Notably, while both chloroquine and hydroxychloroquine can suppress the virus in a laboratory setting, their clinical efficacy is quite limited (Esposito *et al*. 2011; Sheahan *et al*. 2017; Arshad *et al*. 2020; Guy *et al*. 2020; Wang *et al*. 2020). Similar results were reported for a spectrum of other antiviral agents (Sheahan *et al*. 2020). For example, remdesivir (a monophosphoramidate prodrug), which received emergency-use authorization (EUA) from the U.S. Food and Drug Administration (FDA), has demonstrated only marginal benefits in affecting COVID-19 disease outcomes that include survival (Holshue *et al*. 2020; Wang *et al*. 2020; Wang *et al*. 2020; Wang *et al*. 2020). The sole reported benefit for this viral RNA-dependent RNA polymerase (RdRp) inhibitor is reduction of times patients spend in an intensive care unit setting. As a consequence, the FDA withdrew support for its use (FDA 2020). As of November 19, 2020, an EUA was granted only for remdesivir used in combination with baricitinib (a janus kinase inhibitor) to treat hospitalized patients who require supplemental oxygen (Kalil *et al*. 2020). Arbidol (umifenovir), an indole-based anti-influenza drug, is a SARS-CoV-2 therapeutic candidate for use as a pre-exposure prophylaxis for health care workers that may be exposed to SARS-CoV-2 (Vankadari 2020). Arbidol inhibits SARS-CoV-2 by blocking the trimerization of the spike protein (S) and inhibiting the interaction between the virus and a cell's ACE2 receptor with evidence reported of reduced mortality and higher rates of hospital discharge. Collectively, the therapeutic data sets when, taken together underscore the importance of simple virus preventive measures that include social distancing, quarantines, personal protective equipment, and lockdowns (in some extreme cases), all of which highlight the immediate need for widespread vaccinations.

Throughout human history of scientific research, vaccines have been one of the most decisive intervention achievements for the treatment and prevention of infectious diseases. Vaccine development represents a linear multiphase activity. Often the timeline is measured in years, which include identifying a vaccine candidate to accomplishing safety checks, testing efficacy and developing large-scale production (Lurie *et al*. 2020). Thus, formulating an effective vaccine for any given virus is an extensive process. Given vaccine urgency to curb the COVID-19 pandemic, scientists from the academic and biotechnology industry are operating under a collective effort to make vaccines available for public distribution. This occurred in almost unprecedented time spans (Mullard 2020; Thanh Le *et al*. 2020; Li *et al*. 2021). The parameters for vaccine administration remained on ease of administration; facile production and scale-up; long-term storage; and safety and efficacy. Notably and during this pandemic, the compressed timeline generated concern for vaccine viability (Gouglas *et al*. 2018; Excler *et al*. 2021). Nonetheless, computational biology, structure-based antigen design, gene synthesis, and protein engineering accelerated design, facilitated new manufacturing platforms (Anasir and Poh 2019; Corbett *et al*. 2020; Shin *et al*. 2020; Kangabam *et al*. 2021) and accelerated vaccine development and production (Brisse *et al*. 2020).

The rapid development of SARS-CoV-2 vaccines was enabled by immunopathogenic knowledge of viral proteins and its known antibody responses. Moreover, the evolution of next-generation platforms facilitated manufacturing (Heaton 2020). However, each of the past phase III studies have fallen short in establishing optimal efficacy and safety (Musuamba *et al*. 2017). Two of the current vaccines are mRNA-based which must be kept in a deep freeze prior to use (Crommelin *et al*. 2021). Such resources are not commonly available in rural hospitals or urgent care clinics. Whether the vaccines will confer long-term protection against SARS-CoV-2 remains a concern. An additional concern is the antibody-dependent enhancement and accelerated cellular immunopathology that could occur after vaccination. Vaccination could also increase transmission as vaccinated individuals may operate with risk aversion. The vaccine developers also indicated that it is important to evaluate a range of

doses to determine the best dosing regimen for vaccine candidates (Heaton 2020). Another concern in vaccine development is that elderly people are at a greater risk for infection based on weaker vaccine-induced immune responses (DiazGranados *et al.* 2014; Pagliusi *et al.* 2020). Despite the challenges posed by this rapidly spreading virus, there has been an unprecedented level of engagement and cooperation within the scientific community. Safe and effective vaccines will cause a paradigm shift in returning to pre-pandemic normalcy. As of April 9 2021, the COVID-19 vaccine development includes more than 270 candidates. Of those candidates, 86 have currently moved into clinical development. Among various underdeveloped platforms, the most likely to process through are DNA- and RNA- based platforms, which are followed by recombinant subunit vaccines. These platforms are each discussed in the context of virus-induced immune responses from early cell entry to systemic virus-immune engagements.

VIRUS-CELL CROSSTALK

Angiotensin converting enzyme 2 (ACE2), the transmembrane serine protease 2 (TMPRSS2), and pyrin domain-containing protein 3 (NLRP3) viral receptors

ACE2 is a membrane protein located on the surface of the lung, heart, central nervous system, gastrointestinal system, and kidney (Gheblawi *et al.* 2020). The external component of ACE2 is cleaved by sheddases (membrane-bound enzymes that cleave extracellular transmembrane proteins) then released into the systemic circulation. The cleaved portion of ACE2 is the portion that regulates body homeostasis including the regulation of blood pressure, wound healing, and inflammation (Gheblawi *et al.* 2020). ACE2 interacts with angiotensin II and converts it into angiotensin (1-7). Angiotensin is an antioxidant and vasodilator that regulates adrenal angiotensin II.

SARS-CoV-2 accesses its targets through metallocarboxyl peptidase ACE2 receptor that is present on lung and intestinal epithelial cells (Wang, Liu and Gao 2020). ACE2 is used by the SARS-CoV-2 for cell attachment and entry (Donoghue *et al.* 2000; Turner *et al.* 2002; Li *et al.* 2003). The spike S protein protrudes from the surface of the virus. It is assembled as a trimer that consists of two protein subunits, S1 and S2 (Ke *et al.* 2020). The S1 head contains the receptor binding domain (RBD), which directly binds the peptidase domain (PD) of ACE2 to gain entry into host cells (Yan *et al.* 2020). The S2 mediates endocytosis by facilitating membrane fusion. After attachment of the S protein to ACE2 receptor, the S protein undergoes priming by endosomal cysteine proteases, cathepsin B and L (CatB and CatL) (Simmons *et al.* 2005), and a transmembrane serine protease 2 (TMPRSS2) (Glowacka *et al.* 2011). TMPRSS2 is essential for infection, whereas CatB and CatL activity are not (Iwata-Yoshikawa *et al.* 2019). ACE2 binds the viral S protein in its upright position, thereby leading to structural changes that are required for two proteolytic cleavages. The S1-ACE2 interaction triggers the exposure of an S2 cleavage site that will be cleaved by host proteases. All such actions are required for membrane fusion and the endocytosis of the SARS-CoV-2-ACE2 receptor complex (Hamming *et al.* 2004; Wang *et al.* 2008; Shang *et al.* 2020).

Host proteases, such as cathepsins, transmembrane TMPRSS2, TMPRSS4, or human airway trypsin-like proteases (Hoffmann *et al.* 2020), are responsible for the acid-dependent proteolytic cleavage of the S1 viral protein. The exact protease for such activity has not yet been identified. Fusion of the viral and cellular membrane follows proteolytic cleavage. First, cleavage of the viral S protein occurs at two different sites. Second, the initial cleavage separates the RBD and the S protein's fusion domains, whereas the second cleavage exposes the fusion peptide (a cleavage at the S2 protein) (Belouzard, Chu and Whittaker 2009). Third, the binding of the S1 protein to the ACE2 receptor leads to its cleavage at the disintegrin and metallopeptidase domain 17/tumor necrosis factor-converting enzyme. These are operative at the ectodomain sites (Lambert *et al.* 2005; Heurich *et al.* 2014; Oarhe *et al.* 2015). Lastly, TMPRSS2 cleaves ACE2 at the intracellular C-terminal domain (Heurich *et al.* 2014; Hoffmann *et al.* 2020). Disintegrin, metallopeptidase domain 17, and TMPRSS2 facilitate host cell viral entry by endo- and ectodomain cleavage.

The viral S protein contains a polybasic furin cleavage site (PRRAR), which is crucial for infection (Cantuti-Castelvetri *et al.* 2020; Vankadari 2020; Walls *et al.* 2020; Peacock *et al.* 2021; Raghuvamsi *et al.* 2021). PRRAR promotes viral infection and cell-cell fusion (Daly *et al.* 2020; Papa *et al.* 2021). Even in the absence of furin cleavage, infection occurs albeit being reduced (Papa *et al.* 2021). The host protease furin cleaves the full-length precursor viral S protein into two associated polypeptides: S1 and S2. The C terminus of the S1 protein generated by furin cleavage has an amino acid sequence (^{682}RRAR^{685}) that conforms to a [R/K]XX[R/K] motif, termed the "C-end rule" (CendR) (Teesalu *et al.* 2009). The cleavage of the viral S protein to S1, which consists of a polybasic Arg-Arg-Ala-Arg carboxyl-terminal sequence, promotes binding to cell surface neuropilin-1 (NRP1) and NRP2 receptors. NRP1 and NRP2 are transmembrane receptors that regulate axon guidance, angiogenesis, and vascular permeability (Teesalu *et al.* 2009; Plein, Fantin and Ruhrberg 2014). This occurs through internalization of CendR ligands through an endocytic process resembling micropinocytosis (Teesalu *et al.* 2009; Pang *et al.* 2014). Interaction between S1 and CendR motif generated by the furin cleavage of S1/S2 with NRPs can promote entry and infection by SARS-CoV-2. Studies have shown upregulation of NRP1 and NRP2 in COVID-19-diseased lung tissue (Ackermann *et al.* 2020). A natural deletion of the S1/S2 furin cleavage attenuates pathogenicity in animal models of human disease (Lau *et al.* 2020).

Following SARS-CoV-2 cell entry, the virus hijacks ribosomal assembly for its translation. Structural and non-structural proteins are synthesized to produce the replication transcription complex that transcribes viral positive ssRNAs into negative ssRNAs with subgenomic mRNAs. All mRNAs are further translated into structural and accessory proteins that include the membrane, spike, and envelope proteins. Each are transported to the endoplasmic reticulum-golgi intermediate compartment for assembly into the viral genome. They are then complexed with the viral nucleocapsid protein, which is the most abundant protein in the coronavirus. The nucleocapsid protein is a highly immunogenic phosphoprotein, and the nucleocapsid protein is normally very conserved in the RNA sequence. Translation occurs in small vesicles that are released into exocytic vesicles, which then bud from the cell surface as complete virions (Astuti and Ysrafil 2020). SARS-CoV-2 induces cell death through pyroptosis (Vabret *et al.* 2020), a form of inflammatory cell death (Danthi 2016).

NLRP3

Pathogen-associated molecular patterns (PAMPs) affect innate immunity. This is operative in a nonspecific manner upon exposure to infectious agents or cellular injury. The NLRP3 protein (previously known as NACHT, LRR and PYD domains-containing

protein 3 [NALP3] and cryopyrin) is expressed in macrophages as part of the inflammasome (da Costa *et al.* 2019). NLRP3 is involved in pathogen clearance and, following its stimulation, oligomerizes through NACHT domains and recruits an adaptor protein. This is an apoptosis-associated speck-like protein containing a caspase recruitment domain that serves to control innate immune clearance. Mechanistically, processes proceed through homotypic pyrin interactions. The binding of the intermediate complex recruits effector proteins and pro-caspase-1, thereby leading to the NLRP3 inflammasome and the control of pathogen growth and transmission (Swanson, Deng and Ting 2019). PAMPs, which include viral RNAs, are detected by various RNA-sensing systems, such as the Z-DNA binding protein 1, DDX58/RIG-I, and mitochondrial antiviral signaling proteins (Yap, Moriyama and Iwasaki 2020). Mitochondrial antiviral signaling proteins present on the outer membrane of mitochondria induce oligomerization of NLRP3. Thus, intracellular systems interact with each other to induce an inflammasome following cell activation through the viral RNA (Park *et al.* 2013).

SARS-CoV-2 induces NLRP3 inflammasome activation by disrupting Ca^{2+} and K^{+} homeostasis (Shah 2020). As high and low cytosolic concentrations of Ca^{2+} and K^{+}, respectively, activate the NLRP3 inflammasome, envelope (E) protein lodges into the endoplasmic reticulum-golgi intermediate compartment/Golgi membrane. It does so to act as a Ca^{2+} channel to facilitate influx of Ca^{2+} while the open reading frame 3a (ORF3a) protein of SARS-CoV-2 mediates K^{+} efflux (Chen *et al.* 2019). The imbalance of these ions produces reactive oxygen species, which are recognized as damage-associated molecular patterns (DAMPs), and thereby activate the NLRP3 inflammasome. The ORF8b transcript of SARS-CoV translates into polypeptides that form intracellular aggregates. These bind to the leucine-rich repeat domain of the NLRP3 to induce inflammasome assembly (Shi *et al.* 2019). ORF8 encodes a single polypeptide in SARS-CoV-2 that lacks the aggregation motif found in SARS-CoV. ORF8 of SARS-CoV-2 affects immune evasion by inhibiting antigen presentation through major histocompatibility complex-I (MHC-I) (Zhang *et al.* 2020). The ORF3a of SARS-CoV-2 differs from SARS-CoV by likely acting as a K^{+} ion channel in order to induce the NLRP3 inflammasome (Issa *et al.* 2020; Kikkert 2020; Ren *et al.* 2020). Inflammasomes trigger the auto-cleavage of pro-caspase-1 into active caspase-1, and as such, mediate the proteolytic cleavage of pro-interleukin-1beta (pro-IL-1β), pro-IL-18 and the pyroptotic factor gasdermin D into IL-1β, IL-18 and N terminal of gasdermin D, respectively.

The N terminal of gasdermin D induces pyroptosis by embedding into the lipid bilayer. This causes perforations in the cell membranes, thereby leaking the cellular contents into extracellular space. The result is increased levels of IL-18, PAMPs, and DAMPs, which collectively mark the initiation of inflammation (Zhao and Zhao 2020). IL-1β is a pro-inflammatory cytokine that attracts and activates immunocytes from blood to the infection site (Niu *et al.* 2019). Increased levels of IL-1 are linked to lymphopenia in COVID-19 patients and suggest an ongoing inflammation-mediated pyroptosis (Yang 2020). Thus, SARS-CoV-2 infection leads to activation of NLRP3 inflammasome, which serves as a trigger of the cytokine "storm" and risk factor for cell and tissue damage resulting from persistent viral infection (Fig. 1).

Cellular chemotaxis

IL-1β and IL-18 released from virus-infected cells recruit leukocytes at the infection site (Coperchini *et al.* 2020). Neutrophils are the first cells present at the site of infection in response to pro-inflammatory cytokines and chemokines. This includes, but is not limited to, IL-1β activated through the IL-1 receptor. Once activated, neutrophils may affect viral clearance through phagocytosis, degranulation, oxidative burst, and neutrophil extracellular traps (NETs) (Camp and Jonsson 2017). They further release certain IL-6, IL-12, transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), prostaglandin E_2 (PGE2) and leukotriene B4. These effector molecules recruit additional immunocytes like molecular patterns, which further secrete inflammatory mediators (Lehman and Segal 2020). Infiltration of neutrophils into pulmonary capillaries and alveolar spaces has been reported in COVID-19 patient tissues (Fox *et al.* 2020). A positive feedback loop is established with neutrophils recruited in response to IL-1β.

Immune responses observed in COVID-19 arise, in part, from NETs, which are composed of chromatin and proteins that release neutrophils upon their rupture. NETs are detected in blood of patients with the acute respiratory distress syndrome (ARDS) whose levels correlate with disease severity. NETs are linked to the pathogenesis of ARDS, and subsequent organ failure and mortality is observed as a consequence of NET-induced inflammation (Barnes *et al.* 2020). IL-1β activates macrophages and epithelial cells through IL-1R signaling and induces the expression and production of IL-6 and IL-12 (Rathinam and Fitzgerald 2010). IL-1β dendritic cell (DC) stimulation increases interferon (IFN) expression by inducing IFN-stimulated gene expression in response to infection (Aarreberg *et al.* 2018). Both IL-1β and IL-18 released in the early stages of inflammation influence the polarization of T_H cells at the time of infection. IL-1β induces a T_H17 response by inhibiting T_H1 activities, whereas IL-18 activates the T_H1 response (van de Veerdonk *et al.* 2011). T_H17 responses are beneficial for viral replication as it inhibits apoptosis of virus-infected cells by producing Bcl-xL and Bcl-2 (Kim *et al.* 2012). Activation of the T_H17 adds to the inflammatory response by releasing IL-17, which further induces IL-1, IL-6, IL-15 and TNF-α (Pacha, Sallman and Evans 2020). An increased numbes of T_H17 cells seen in blood samples of COVID-19 patients with increased cytokine levels support a role of T_H17-mediated inflammation in disease (Wu and Yang 2020). IL-18 also plays a pathogenic role in disease, as it modulates natural killer cell (NK), DC, macrophage, T cell, and B cell activities. IL-18 is a pro-inflammatory cytokine regulating IFN-γ in NK and T cells with IL-12. Its pro-inflammatory effect follows TNF-α, nitric oxide, and prostaglandin induction. In synergy with IL-12, IL-18 induces a T_H1 response. However, without IL-12, a T_H2 response is initiated (McInnes *et al.* 2000). The cytokine profile that is generated during an infection determines the type of T_H cells that are activated. The T_H1 response is seen through pro-inflammatory cytokines IL-1, IL-2, IL-12, TNF-α and IFN-γ.

In contrast, T_H2 responses, which are indicative of extracellular pathogens, produce an anti-inflammatory cytokine profile that includes IL-4, IL-5, IL-6 and IL-10 (Costela-Ruiz *et al.* 2020). For antiviral immunity Fas ligand, T- and NK cell activities occur in coordination with the upregulation of granulocyte macrophage colony-stimulating-factor (GM-CSF) and IL-10 through IL-18. IL-18 induces release of IL-8, MIP-1α, MIP-1β and MCP-1, which recruit monocyte-macrophages to the site of infection (Pirhonen 2001).

After the death of virus-infected cells, DAMPs and PAMPs appear in the extracellular space, which are then recognized by extracellular and endosomal pattern recognition receptors (PRRs) on immunocytes. One such pattern recognition receptor is the Toll-like receptors (TLRs), which are expressed on DCs,

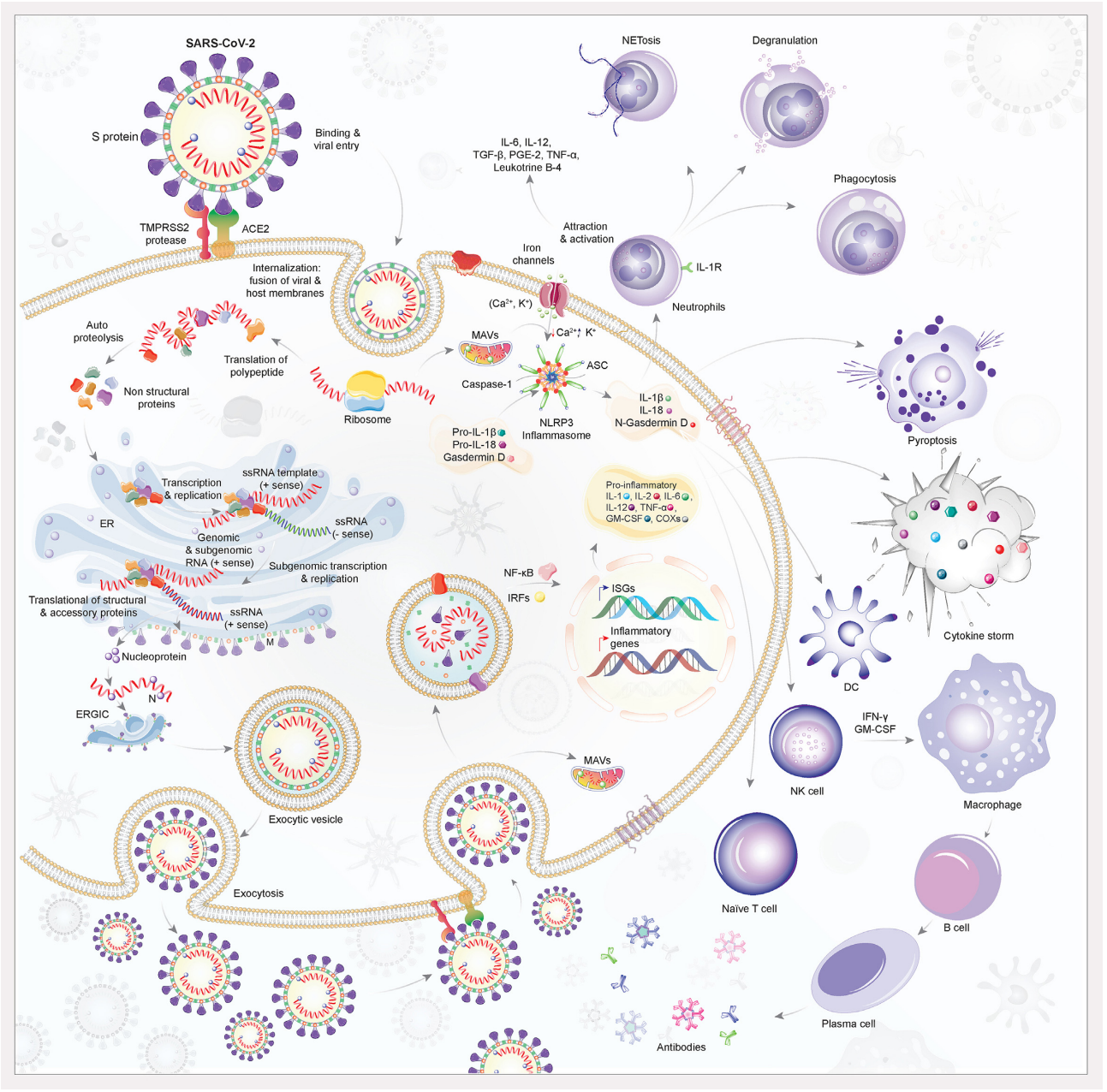

Figure 1. Virus infection induces host immune responses. Schematic representation of the genomic and sub-genomic organization of SARS-CoV-2. During the viral replication cycle, SARS-CoV-2 first binds to the ACE2 receptor, which engages TMPRSS2 for entry into an epithelial host cell. Following entry of the genomic RNA into cell cytoplasm, the two large open reading frames (ORFs) 1ab are translated into a viral transcriptase complex (phosphatase activity and RNA-dependent RNA polymerase (RdRp) and a helicase). Replication of the genome involves the synthesis of a full-length negative-strand RNA and serves as a template for full-length genomic RNA. After translation, structural proteins are localized to the golgi intracellular membranes and the endoplasmic reticulum golgi intermediate compartment where budding occurs. New virions that are assembled with full genome RNA are released from the cell by exocytic vesicles. Oligomerization and activation of NLRP3 inflammasome are illustrated through different pathways. MAVs serve to recognize pathogen-associated DNA/RNA which transcend a signal. Calcium potassium imbalance contributes to inflammasome activation. PAMPs and DAMPS, produce active IL-1β, IL-18, and N-Gasdermin D. These engage the innate and adaptive immune systems(figure originally made in house by authors).

NKs, neutrophils, macrophages, T cells and B cells (Leifer and Medvedev 2016). Different molecular patterns stimulate different TLRs. TLR3, TLR7, and TLR8 are known to be stimulated by PAMPs raised during a viral infection (Kawasaki and Kawai 2014; Kikkert 2020). TLRs are linked to viral clearance failures, and as such, lead to ARDS and other life-threatening clinical disease manifestations. The TLR family is comprised of ten members (from TLR1–TLR10). These are expressed by macrophages, epithelia, and fibroblasts. Activation of TLRs occurs through PAMPs, thereby leading to the production of inflammatory cytokines and type I IFNs. TLR-1, -2, -3, -4, -5, -6, -10 are present on the cell surface or the endosomal compartments (TLR-3, -7, -8, -9) (O'Neill, Golenbock and Bowie 2013). TLR3 is present on both the cell surface and the cell endosomal compartments (Matsumoto *et al.* 2013). Similar to SARS-CoV, SARS-CoV-2 may alter the emergence of effective antiviral immune responses.

This is seen in more severe COVID-19 disease by inhibition of the TNF-receptor-associated factors (TRAF) -3 and -6. These play an essential role in inducing IFN regulatory factor-3/7 in response to TLR activation. Agonists for TLR-7 may prevent severe COVID-19 disease and boost antiviral responses (Kawasaki and Kawai 2014). TLR7 and TLR8 work through their recognition of ssRNA predominantly expressed in plasmacytoid and myeloid DCs (Saitoh *et al.* 2017; Meas *et al.* 2020). Stimulation of TLR3 by dsRNA leads to the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and IRF3. This is specifically accomplished by the TLR domain-containing adapter-inducing IFN (TRIF)-modulated signaling. TLR7 and TLR8 induce the MyD88/IRAK-4/TRAF-6 signaling, which activates IκB kinase-ε complex and MAPK kinases, thereby leading to NF-κB activation (Barton and Kagan 2009). In turn, activation of NF-κB leads to downstream expression of pro-inflammatory cytokines IL-1, IL-2, IL-6, IL-12, TNF-α, GM-CSF, IFNs, and cyclooxygenase (Zhang and Ghosh 2001). The genes responsible for such cytokines are also induced by IL-1β and IL-18R signaling. These cytokines exert their inflammatory responses by directly damaging the cells, and by further recruiting and activating more immune cells, which in turn, produce more pro-inflammatory cytokines.

Alveolar macrophages express ACE2 receptors, thereby making them potentially susceptible to SARS-CoV-2 infection. Large numbers of activated macrophages aggregate in lung tissue during advanced COVID-19 disease and play a pivotal role in the development of ARDS. This is due to macrophage polarization (Wang *et al.* 2020). $ACE2^+$ $CD169^+$ macrophages exposed to SARS-CoV-2 harbor the nucleocapsid protein found in virus-exposed cells (Feng *et al.* 2020). $CD169^+$ macrophages are also present in lymph nodes and spleen. The upregulated expression of Fas and FasL may indicate involvement of activation-induced cell death of T and B cells, to account for lymphocytopenia seen in COVID-19 patients. Nonetheless, abortive infection of macrophages is sufficient to induce a cytokine storm (Nguyen and Russell 2001; Jafarzadeh *et al.* 2020). Indeed, virus-induced activation and consequent inflammation ensues as a result of virus-macrophage interactions. Macrophages are a major antigen-presenting cell that presents peptide antigen in the context of MHC-I and MHC-II molecules (Wieczorek *et al.* 2017). Macrophage activation is linked to inflammation and tissue damage. This is due to the presence of SARS-CoV-2 in the absence of productive infection and a linkage between high levels of IL-6 and lower numbers of blood $CD4^+$ and $CD8^+$ T cells.

Antibody-dependent enhancement of viral infection

A few months after the onset of the COVID-19 pandemic, children with multisystem inflammatory syndrome were reported (Hoste, Van Paemel and Haerynck 2021). There were 14 reported cases of children under the age of 10 that had multisystem inflammatory syndrome among the total of 8,866 cases of COVID-19 in a single Chinese study. In addition, the number of cases in children aged 10 to 19 was 1% (She, Liu and Liu 2020; Wu and McGoogan 2020). This was of interest, as the incidence of Kawasaki disease is reported to be higher in Asian populations (Rowley and Shulman 2018). Notwithstanding, systemic inflammation in COVID-19 pediatric cases was rarely observed (Wu *et al.* 2020). This could reflect the low number of pediatric COVID-19 patients. The percentage of asymptomatic SARS-CoV-2 infection in children and multisystem inflammatory syndrome suggests a role of nonspecific antibodies in disease development. This could occur through antibody-dependent enhancement (ADE). Indeed, ADE develops when non-neutralizing viral antibodies enhance infection by affecting entry and cell replication (Iwasaki and Yang 2020). Non-neutralizing antibodies can arise either in response to SARS-COV-2 infection or may arise due to a Hoskins effect (Fierz and Walz 2020). Hoskins effect occurs when the immune system engages immunological memory that is based on a prior viral infection and after being re-exposed to a different or cross-reactive pathogen. Shared protein homology between SARS-CoV-2 and other human coronaviruses points to cross-reactive antigens. When SARS-CoV-2 infects an individual with an immunological memory of these cross-reactive antigens, the host defense system mounts a secondary immune response. This results in antibody production that can lead to enhanced infection. The development of ADE depends on the exposure of cross-reactive antigens across viral groups. Serological surveys in viral samples of COVID-19 need to be conducted to assay for ADE in relationship to disease severity (Kumar *et al.* 2020; Lee *et al.* 2020).

A well-known mechanism for ADE is endocytosis of the virus-antibody complex (Iwasaki and Yang 2020). Endocytosis takes place when the non-neutralizing antibody binds Fc receptors present on the surface of an immune cell, which increases infection and viral replication (Haslwanter *et al.* 2017). Coronaviruses show that ADE develops independent of cross-reactive immune response. Neutralizing antibodies raised against an S protein of specific viral strains can also mediate ADE by binding and stabilizing the S protein and binding the Fc receptors to aid viral entry (Wan *et al.* 2020). The association of ADEs with COVID-19 disease severity is a putative warning for vaccine-related protective responses. Currently, 45 vaccine candidates based on different platforms have been granted U.S. FDA fast track and safety evaluation that include testing for ADE to weigh the benefits against untoward effects (Fauci, Lane and Redfield 2020).

SARS-CoV-2 AND IMMUNE RESPONSES

Innate immunity

Innate immune responses are nonspecific, providing immediate, non-targeted defense against infection, whereas the adaptive immune responses offer a more targeted, long-lasting response acquired over time. The innate immune system's macrophages and DCs sense pathogens and contain them, thereby serving as the first line of antiviral defense. There is limited understanding of precise innate immune responses against SARS-CoV-2; however, the virus-host interactions involving SARS-CoV-2 are likely similar to those of other coronavirus strains, given the shared sequence homology among coronaviruses and the conserved mechanisms of innate immune signaling (Vabret *et al.* 2020; V'Kovski *et al.* 2020). Virus-derived ssRNA and dsRNA serve as PAMPs that engage and activate pattern recognition receptor on the innate immune cells in support with cytosolic RIG-I like receptors (RLRs) and extracellular and endosomal TLRs. Upon pattern recognition receptor activation, downstream signaling cascades trigger the secretion of cytokines, including type I/III IFNs, TNF-α, IL-1, IL-6, and IL-18. Together, these cytokines induce antiviral programs into the specific target cells and potentiate adaptive immune responses (Vabret *et al.* 2020).

Type I IFNs can effectively limit coronavirus infection if present early and localized properly (Channappanavar *et al.* 2016; Channappanavar and Perlman 2017; Channappanavar *et al.* 2019). Early evidence demonstrated that SARS-CoV-2 is more sensitive to both type I and III IFN pretreatment *in vitro* than SARS-CoV (Blanco-Melo *et al.* 2020; Lokugamage *et al.* 2020; Mantlo *et al.* 2020; Stanifer *et al.* 2020). However, the specific

IFN-stimulated genes that mediate such protective effects are still being elucidated. Lymphocyte antigen-6 complex locus E (LY6E) has been shown to interfere with SARS-CoV-2 S protein-mediated membrane fusion (Pfaender *et al.* 2020; Wei *et al.* 2020). It is likely that IFN-induced transmembrane family of proteins may inhibit SARS-CoV-2 entry as demonstrated for SARS-CoV (Huang *et al.* 2011; Zhao *et al.* 2014; Zhao *et al.* 2018).

Neutrophils

Neutrophils are active in early innate nonspecific protective immune responses to viral infections. This is most relevant to the upper respiratory tract. Through degranulation and lysis, they can be protective to limit infection while also being cytotoxic and speeding severe pneumonia that follows the initial coronavirus infection (Haick *et al.* 2014; Camp and Jonsson 2017). Neutrophils can also exaggerate lung inflammation caused by the influenza virus. From the COVID-19 literature, an increased peripheral neutrophil-to-lymphocyte ratio was associated with poor prognosis (Zheng *et al.* 2020). However, the mechanisms of how neutrophils affect SARS-CoV-2-infected lungs are not well understood. COVID-19 lung damage in some patients might involve dysregulated neutrophil activity (Didangelos 2020). Whether or not dysregulated neutrophil activity is associated with COVID-19 lung damage is difficult to determine given the convolution of innate immune responses and the importance of neutrophils in initial antiviral defense as well as their role in secondary bacterial and fungal infections that are common comorbidities in COVID-19 patients. Universal control of innate immunity underlies the clinical effectiveness for the use of steroids for COVID-19 disease. It is certainly believed possible to target specific inflammatory mechanisms to avert lung injuries or systemic hyperinflammation and cytokine storms. The target mining existing here includes multiple druggable proteins such as neutrophil-attracting chemokine signaling, neutrophil-relevant inflammatory entities, and SARS-CoV-2 receptors. The immunopathobiological mechanisms driving COVID-19 is certainly evolving in our understanding of virus-mediated pathologies (Didangelos 2020).

Natural killer cells (NK)

NK cells are innate effector lymphocytes. Evidence for an immediate role of NK cells in protection against viral infections comes from patients with selective NK cell deficiencies, as this group develops fulminant viral infections, with herpes virus infections being a prominent example (Wilk *et al.* 2020; Zheng *et al.* 2020). Human NK cells have also been shown to rapidly respond during the acute phase of infections in humans with hantavirus, tick-borne encephalitis virus, influenza A virus, dengue virus and after vaccination with live-attenuated yellow fever. NK cells not only have the capacity to target and kill infected cells directly, but they could also influence adaptive T lymphocyte responses (Zheng *et al.* 2020). The step of NK cell activation can function as a rheostat in regulating T cells. A particular level of NK cell activation might promote infection control, while another degree of activation may render immunopathology (Vabret *et al.* 2020; Wang *et al.*2020). Compared to peripheral blood lymphocytes, the human lung is more enriched in NK cells exhibiting $CD56^{dim}$ phenotype which have the capacity to respond to viral infections, like the influenza A virus (Jegaskanda *et al.* 2013; Björkström, Ljunggren and Michaëlsson 2016). Analysis of ventilator-dependent COVID-19 patient samples showed a substantial decrease in $CD56^{dim}$ NK cells, a primary contributor towards host antiviral immunity through cell-mediated cytotoxicity. $CD56^{bright}$ NK cells, considered robust producers of IFN-γ and tumor necrosis factor α, were observed to be significantly depleted in COVID-19 samples (Wilk *et al.* 2020). Low peripheral blood NK cell numbers have also been observed in this disease. Two studies assessing the single-cell landscape of immune cells within the bronchoalveolar lavage fluid of COVID-19 patients have suggested that NK cell numbers increase at this site of infection. Given the leading important role of NK cells in acute viral infections, their relative substantial presence within lung tissue, and their associations with immunopathology, underscore their possible role(s) in COVID-19 disease (Hadjadj *et al.* 2020; Wang *et al.* 2020).

Macrophages

Macrophages are innate immune cells that encounter invading pathogens. Macrophages sense their environment and phagocytose pathogens that would be harmful to the organism (Gendelman *et al.* 1989). Other functions of macrophages include intracellular killing, antigen presentation, secretory activities, and process mobility (Merad and Martin 2020). Therefore, macrophages induce a nonspecific immune response against invading pathogens. Monocytes are bone marrow-derived leukocytes, which travel in the blood and spleen and are characterized by their ability to recognize “danger signals” via pattern recognition receptors. Monocytes can phagocytose, present antigens, secrete chemokines, and proliferate in response to infection and injury. Once recruited to tissues, monocytes are capable of differentiating into macrophages and DCs (Cheung *et al.* 2005; Chiu and Bharat 2016). Macrophages are terminally differentiated cells that phagocytose pathogens, debris, or toxins. When activated, they secrete factors that include cytokines and chemokines, thereby enabling the recruitment of other immunocytes. They, along with DCs, migrate to secondary lymphatic tissues where they present processed antigens (Hirayama, Iida and Nakase 2017).

Several mechanisms affect macrophage hyperactivation as seen in patients with COVID-19. Late production of IFN-1-stimulating cytopathic effects and expanded detection of microbial risks promote the enhanced release of monocyte chemoattractants by alveolar epithelial cells (and likewise by macrophages and stromal cells), thereby leading to sustained recruitment of blood monocytes into the lungs. Monocytes differentiate into macrophages that transition to pro-inflammatory or regulatory states after activation depending on their environmental conditions. The former occurs through the activation of Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathways. Activated NK cells and T cells further promote the recruitment and activation of monocyte-derived macrophages through the production of GM-CSF, TNF-α, and IFN-γ. Oxidized phospholipids collect in infected lungs and activate endothelial cells, which recruit and bind to monocytes. Oxidized phospholipids trigger macrophage activation through the TLR4–TRAF6-mediated NF-κB pathway. It has been shown that TRAF6 binds TRIF, thereby serving as a mediator of TRIF-mediated activation of NF-κB (an innate immunity pathway). Interfering with monocytes and endothelial cell activation in response to oxidized phospholipids could prevent thrombotic complications in people who have pre-existing cardiovascular and metabolic comorbidities. This includes, but is not limited to, those that suffer from COVID-19 virus sensing. This would trigger TLR7 activation through a viral single-stranded RNA recognition pattern. It is also possible that type I IFNs induce the

expression of SARS-CoV-2 entry receptors. Human monocytes and macrophages express ACE2 as well as TMPRSS2 and furin, which appear to be a widespread target for SARS-CoV-2 infection (Abassi *et al.* 2020; Wang *et al.* 2020).

The virus gains access to the cytoplasm of macrophages to activate the NLRP3 inflammasome, which leads to the secretion of mature IL-1β and/or IL-18. IL-1β, amongst other pro-inflammatory cytokines, amplifies activation of macrophages in an autocrine or paracrine fashion and results in reductions in type I IFN. This is especially notable in infected lungs. The engagement of Fc-γ receptors (FcγRs) by anti-spike protein IgG could contribute to increased macrophage pro-inflammatory activation. Activated macrophages exacerbate the COVID-19 cytokine storm via releasing substantial amounts of proinflammatory cytokines. These include, but are not limited to, the CC-chemokine ligand, the CXC-chemokine ligand 10 (CXCL-10), IFN-stimulated genes, the immunoreceptor tyrosine-based activation motif and the TRIF-related adaptor molecule (Merad and Martin 2020) (Fig. 2).

Dendritic cells (DCs)

Based on the function of DCs in immune surveillance, the priming and tolerance that DCs play are important roles within the immunopathology of SARS. DCs play a pivotal role in both innate and adaptive immune responses. As professional antigen-presenting cells, they represent key components of innate response to pathogen infection and orchestrators of the subsequent adaptive immunity (Campana *et al.* 2020). Immature DCs reside in the respiratory tract for immune surveillance and respond dynamically to local tissue inflammation in airways and distal lungs. C-type lectin receptors and TLRs recognize foreign pathogen conserved patterns that allow the induction of adaptive immune responses. Some TLRs are primarily expressed on the cell surface and differentially by DC subsets to modulate the stimuli (Campana *et al.* 2020). Viral glycoproteins may bind to TLR-2 and TLR-4; ssRNA binds to TLR-7 and TLR-8; dsRNA binds to TLR-3; and viral DNA binds to TLR-9 (Xagorari and Chlichlia 2008). The binding of ligands to TLRs may trigger the downstream signaling pathways that are involved in cytokine release and anti-inflammatory mechanisms. This migration of DCs from peripheral tissues to the lymph nodes is relatively important for antigen presenting cells and the triggering of adaptive immune responses. The triggering of DCs is regulated by chemokines, microenvironment, and chemokine receptors (CCRs). The CCRs are expressed during DC maturation and some viruses, such as coronaviruses and herpes simplex virus, can block CCR expression on DCs. These factors are capable of infecting DCs and facilitate their replication and distributions (Tiberio *et al.* 2018). However, some direct and indirect pieces of evidence suggest that these cells might be involved in the development and evolution of COVID-19 disease, although it is unclear whether DCs might be effectors of SARS-CoV-2 action or targets of virus infections, or both. The lack of chemokine response, intense chemokine upregulation, and induction of CCR expression in SARS-CoV-2-infected DCs suggests unique SARS immunopathology (Chen *et al.* 2020; Lopez-Collazo *et al.* 2020; Vabret *et al.* 2020).

Adaptive immunity

The adaptive immune system is highly antigen-specific and provides long-lasting protection against infectious pathogens. It is capable of creating memory that would protect against future SARS-CoV-2 infections. Understanding the interplay between innate and adaptive immunity is crucial for vaccine development and implementation.

T cells

T cells play a fundamental role in viral infections. $CD4^+$ T cells provide B cells help with antibody production and coordinate the response of other immune cells, whereas $CD8^+$ T cells kill infected cells to reduce the viral burden. It is well established that major histocompatibility complex (MHC)-I and -II molecules are recognized by $CD8^+$ and $CD4^+$ T lymphocytes, respectively. After maturation in the thymus, the T cell expresses a unique cell surface antigen-binding molecule called the T cell receptor (TCR). This antigen-binding molecule consists of two transmembrane molecules, the TCR-α and the TCR-β, that are the result of rearrangement of first, the TCR-β, and then, the TCR-α genes. In contrast to membrane-bound antibodies on B cells which can recognize antigen alone, the vast majority of TCRs recognize a complex ligand that includes an antigenic peptide bound to a MHC-derived molecule. These two major forms of polymorphic membrane-bound glycoproteins exist as MHC-I and - II molecules that interact with $CD8^+$ and $CD4^+$ T cells. Whereas MHC-I molecules are expressed by nearly all nucleated cells, MHC-II molecules are constitutively expressed only by antigen-presenting cells (APCs) (Andersen *et al.* 2006).

The antigen-MHC-II complex is presented to the T cell receptor (TCR) complex present on the surface of naïve T cells. However, a mere interaction between the two does not activate the naïve T cell, as co-stimulatory signals interacting between the surfaces of two cells are required for complete activation of T cells. A classical three-signal hypothesis (MHC, co-stimulation, and cytokines) involves the recognition of a pair of such necessary ligands and co-stimulatory receptors (Goral 2011). B7-1 (CD80) (or B7-2/CD86) is expressed on the surface of APCs and binds with a co-stimulatory receptor, CD28, which is constitutively expressed on the surface of $CD4^+$ and $CD8^+$ naïve T cells. During infection, co-stimulatory molecules can be upregulated. In the absence of CD28, other co-stimulator molecules can prime naïve T cells. This includes CD28-B7-1 functioning as a primary co-stimulation signaling pathway (Goral 2011). Upon activation, naïve T cells proliferate and differentiate into effector cells; how this occurs depends on the type of MHC-peptide complex that is presented, and the cytokines that are produced. Following viral infection, follicular helper T cells (Tfh) are antigen-experienced $CD4^+$ T cells found in the periphery within B cell follicles of secondary lymphoid organs such as lymph nodes, spleen, and Peyer's patches, and are identified by their constitutive expression of the B cell follicle homing receptor CXCR5 (Fazilleau *et al.* 2009). Upon cellular interaction and cross-signaling with their cognate follicular B cells, Tfh cells trigger the formation and maintenance of germinal centers through the expression of CD40 ligand (CD40L) and the secretion of IL-21 and IL-4 (Seo, Youn and Kim 2009). Tfh cells also migrate into these seeded germinal centers, which are predominantly composed of rapidly dividing and mutating B cells. Within germinal centers, Tfh cells play a critical role in mediating the selection and survival of B cells that go on to differentiate into either (i) special plasma cells capable of producing high-affinity antibodies against foreign antigen, or (ii) memory B cells capable of quick immune re-activation in the future (if ever the same antigen is re-encountered). It is possible that Tfh cells might arise as branches in the T_H1 and T_H2 polarized helper cells. These T helper cells proliferate and differentiate into T_H1 cells, which mount an antiviral response and activate macrophages

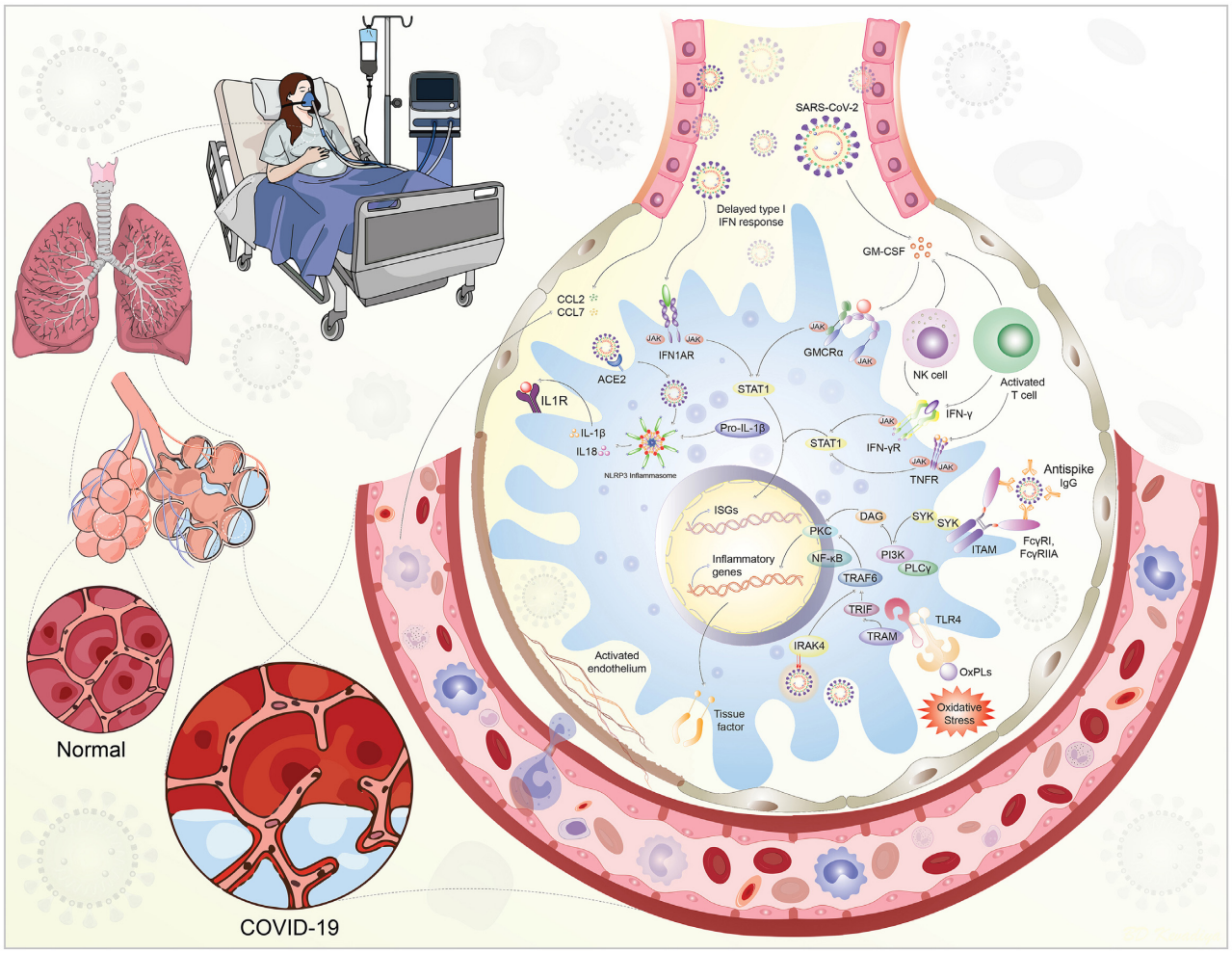

Figure 2. Viral induction of macrophage signaling pathways. SARS-CoV-2 infection of lung epithelial cells leads to induction of expression of CCL2 and CCL7 chemokines, which in turn, affect the release of a cascade of pro-inflammatory factors that include, but are not limited to, alveolar macrophage TNF-α and IL-6. This occurs in parallel with neutrophil chemoattractants, CXCL1 and CXCL2. CCL2 and CCL7 significantly increases the infiltration of leukocytes (macrophages and lymphocytes) with neutrophil accumulation. This leads to activation of macrophages and facilitates cell entry into alveoli through a leaky endothelium. In tandem, type I IFN responses are operative from alveolar epithelial cells leading to activation of the JAK-STAT pathway. This process increases transcription and translation of interferon-stimulated genes and additional inflammatory agents. NLRP3 inflammasome, formed due to infection, converts Pro IL-1β and Pro IL18 into IL-1β and IL-18, respectively, which work in autocrine fashion in both lymphocytes and macrophages. The cytokine GM-CSF further engages the JAK-STAT pathway serving to amplify the release of inflammatory molecules through the GMC receptor, and also by causing lymphocyte release of IFN-γ, TNF-α from activated NK cells and T cells. Phagocytosis of SARS-CoV-2 and oxidative stress causes signaling by TLR2, TLR4 to further drive synthesis of inflammatory molecules via NF-κB mediated protein kinase B signaling. Antibody bound SARS-CoV-2 also adds to this cascade by binding to Fc receptor binding and ITAM and spleen tyrosine kinase-based signaling (figure originally made in house by authors).

and CD8$^+$ T cells into cytotoxic T lymphocytes cells (CTLs) that recognize and kill virus-infected cells by cell-mediated immunity. T_H2 cells that induce B cell activation and proliferation lead to antibody production by humoral immunity along with factors necessary to differentiate T_H1 from T_H2 lymphocyte populations.

Low levels of total T cells in peripheral blood have been reported in COVID-19 patients (Qin *et al.* 2020). Interestingly, increased levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, affect T cell numbers and function. T cell counts are also associated with IL-10, an inhibitory cytokine, that through expression of inhibitory receptors reduces T cell proliferation and promotes T cell exhaustion. This has been shown in COVID-19 patients by increased expression of PD-1 and TIM-3 on T cells and reduced effector T cell numbers, which affect apoptosis (Diao *et al.* 2020). However, the presence of these markers does not definitively prove the exhaustion of T cells. In fact, it has been reported from clinical data that PD-1 expressing SARS-CoV-2 specific cells are functional (Rha *et al.* 2021). Considering all the facts, PD-1 and TIM-3 can definitively be used as a biomarker for disease. Other markers may be looked at, such as NKG2A. Functional exhaustion of CTLs is operative in COVID-19 patients with increased expression of NKG2A, an inhibitory cell surface receptor (Zheng *et al.* 2020). NKG2A recognizes and binds HLA-E complexed with signal peptide MHC-I, which is upregulated in response to IFN-γ (Haanen and Cerundolo 2018).

In patients where the CD4$^+$:CD8$^+$ ratio was unaltered, upregulation of CD8 expression on CTLs was linked to SARS-CoV-2-mediated immune responses (Ganji *et al.* 2020). A contrasting study reports a decreased CD4$^+$:CD8$^+$ ratio due to suppression of CD4$^+$ cells and activation and increase in proliferation of CD8$^+$ cells (Khan *et al.* 2020). In accordance with upregulation of

$CD8^+$ expression, many studies have concluded that SARS-CoV-2 skews the immune response towards a T_H1 response-inducing immunopathology (Grifoni *et al.* 2020; Wei *et al.* 2020); however, COVID-19 patients show a cytokine profile (IL-2, IL-4, IL-7, IFN-γ, IL-10, GM-CSF, IP-10, MCP-1, MIP-1α, and TNF-α) that indicates activation of both T_H1 and T_H2 responses (Zhong *et al.* 2020). In addition to the detection of T_H2 cytokines, cytological characteristics of a T_H2 response such as an increased number of eosinophils, basophils, degranulated eosinophils, plasma cells, or $CD8^+$ T cell lymphopenia were observed in intensive care COVID-19-afflicted patients (Roncati *et al.* 2020). T_H2 response may arise due to high viral titers or pre-existing medical conditions in infected patients, such as cancer, immunodeficiency, autoimmune disorder, and other conditions that may influence failure to mount an effective T_H1 response (Spellberg and Edwards 2001). Further studies are required to resolve the splitting clinicopathological characteristics found in various COVID-19 patients, as the induction of both protective and detrimental T cell responses are crucial vaccine development strategies.

SARS-CoV-2 infections also contribute to an operative dysregulated T cell response (Swain, McKinstry and Strutt 2012; Kalfaoglu *et al.* 2020; Kalfaoglu *et al.* 2021). To best understand this role, two major questions need to be addressed. First, what are the contributions of T cells to viral control in the context of COVID-19? Second, how do memory T cells establish themselves after contributing to protective immunity upon reinfection? Some potential answers are beginning to emerge (He *et al.* 2005). Several reports emphasize the occurrence of lymphopenia with drastically reduced numbers of both $CD4^+$ and $CD8^+$ T cells in moderate and severe COVID-19 cases (Chen *et al.* 2020; Nie *et al.* 2020; Vabret *et al.* 2020). In patients admitted to the intensive care unit, $CD8^+$ T cell lymphopenia seemingly correlated with COVID-19-associated disease severity and mortality (Chen *et al.* 2020; Diao *et al.* 2020; Liu *et al.* 2020). Patients with mild symptoms, however, typically presented normal or marginally higher T cell counts (Liu *et al.* 2020). A number of mechanisms likely contribute to the reduced number of T cells in the blood, including effects from the inflammatory cytokine storm. Indeed, lymphopenia seems to positively correlate with serum IL-6, IL-10, and TNF-α (Diao *et al.* 2020), while patients that received convalescent plasma therapy were found to have restored bulk T cell frequencies in association with lower pro-inflammatory cytokine levels (Diao *et al.* 2020; Liu *et al.* 2020). Cytokines – for example, IFN-γ and TNF-α – may inhibit T cell recirculation in blood by promoting retention in lymphoid organs and attachment to the endothelium. However, an autopsy report examining the spleens and hilar lymph nodes of six patients who succumbed to COVID-19 revealed extensive cell death of lymphocytes and suggest the potential involvement of IL-6 as well as Fas-FasL interactions (Feng *et al.* 2020). In support of this hypothesis, the IL-6 receptor antagonist tocilizumab (TCZ) was found to increase the number of circulating lymphocytes (Giamarellos-Bourboulis *et al.* 2020). T cell enrollment to sites of infection may also decrease their presence in the peripheral blood compartment. Single-cell RNA sequence analysis of bronchoalveolar lavage fluid from COVID-19 patients revealed an increase in $CD8^+$ T cell infiltrate with clonal expansion. Likewise, postmortem examination of a patient who succumbed to ARDS following SARS-CoV-2 infection showed extensive lymphocyte infiltration in the lungs (Tian *et al.* 2020).

SARS-CoV-specific T cell immunity may serve as a guide for further understanding viral-mediated immune alterations. Immunogenic T cell epitopes are distributed across several SARS-CoV proteins (S, N, and M, as well as ORF3) (Machhi *et al.* 2020); however, $CD4^+$ T cell responses were more restricted to the viral S protein. In SARS-CoV survivors, the magnitude and frequency of virus-specific CD8+ memory T cells exceeded that of CD4+ memory T cells, and virus-specific T cells persisted for at least 6–11 years, suggesting that T cells may confer long-term immunity. Overall, patients recovering from COVID-19 demonstrated robust $CD4^+$ and $CD8^+$ T cell responses, but a higher proportion of $CD8^+$ T cells was seen in mild disease. Such observations possibly suggest duality of T cells with respect to a protective versus pathogenic response role. However, the quality of $CD4^+$ T cell responses needs to be further characterized to understand their association with disease severity. Few studies thus far have characterized specific T cell immunity in SARS-CoV-2 infection (Braun *et al.* 2020; Giamarellos-Bourboulis *et al.* 2020; Liao *et al.* 2020).

Reports of functional T cell changes associated with COVID-19 are limited. Most preprints and peer-reviewed studies show increased frequency of activated T cells characterized by expression of HLA-DR, CD38, CD69, CD25, CD44, and K_i-67 in COVID-19 patients (Schulte-Schrepping *et al.* 2020). Elevated levels of activated $CD4^+$ T cells were reported in the hospitalized COVID-19 patients when such were compared to $CD8^+$ T cells (Chen and John Wherry 2020; Mathew *et al.* 2020). $CD4^+$ T cells secreting T_H1, T_H2, and T_H17 cytokines were observed in hospitalized COVID-19 patients (Weiskopf *et al.* 2020). Th1 cells secreting IFN-γ and expressing the T-box transcription factor (TBX21, formerly T-Bet) were elevated in patients with moderate COVID-19 disease compared to mild disease (Chen *et al.* 2020).

In another study, both $CD4^+$ and $CD8^+$ T cell counts were reduced in the blood of patients who died from COVID-19. However, $CCR6^+$ T_H17 pro-inflammatory cell concentrations increased in parallel with elevated CTLs measured in blood from the same patients (Xu *et al.* 2020). TGF-β and IL-6, both of which are produced by $CD4^+$ T cells, are the key cytokines elevated in the patients with COVID-19-associated respiratory compromise (Wang *et al.* 2020; Wei *et al.* 2020).

In general, COVID-19 disease is associated with T cell activation that exhibits trends toward exhaustion based on continuous expression of inhibitory markers, such as PD-1 and TIM-3, as well as overall reduced polyfunctionality and cytotoxicity (Chen and John Wherry 2020). Conversely, recovering patients show an increase in follicular helper $CD4^+$ T cells (Lipsitch *et al.* 2020) as well as decreasing levels of the above inhibitory markers and enhanced levels of effector molecules, such as granzyme A, granzyme B, and perforin. Collectively, these studies provide insights into T cell profiles of acute SARS-CoV- 2 infection (Fig. 3).

B cells

The humoral immune response is critical for clearance of viruses and is a major part of the memory response that prevents reinfection. SARS-CoV-2 elicits a robust B cell response, as evidenced by the rapid and near-universal detection of virus-specific IgM, IgG and IgA, and neutralizing IgG antibodies in the days following infection. The kinetics of such antibody responses to SARS-CoV-2 are now reasonably well-described. In a manner similar to SARS-CoV infection protocol, seroconversion occurs in most COVID-19 patients between 7 to 14 days after the onset of symptoms in which antibody titers persist in the weeks following virus clearance. Antibodies binding the SARS-CoV-2 internal N protein and the external S protein are commonly detected. The RBD of the S protein is highly immunogenic. Potent neutralizing antibodies would bind to the host entry receptor, ACE2, and thus block viral interaction with such

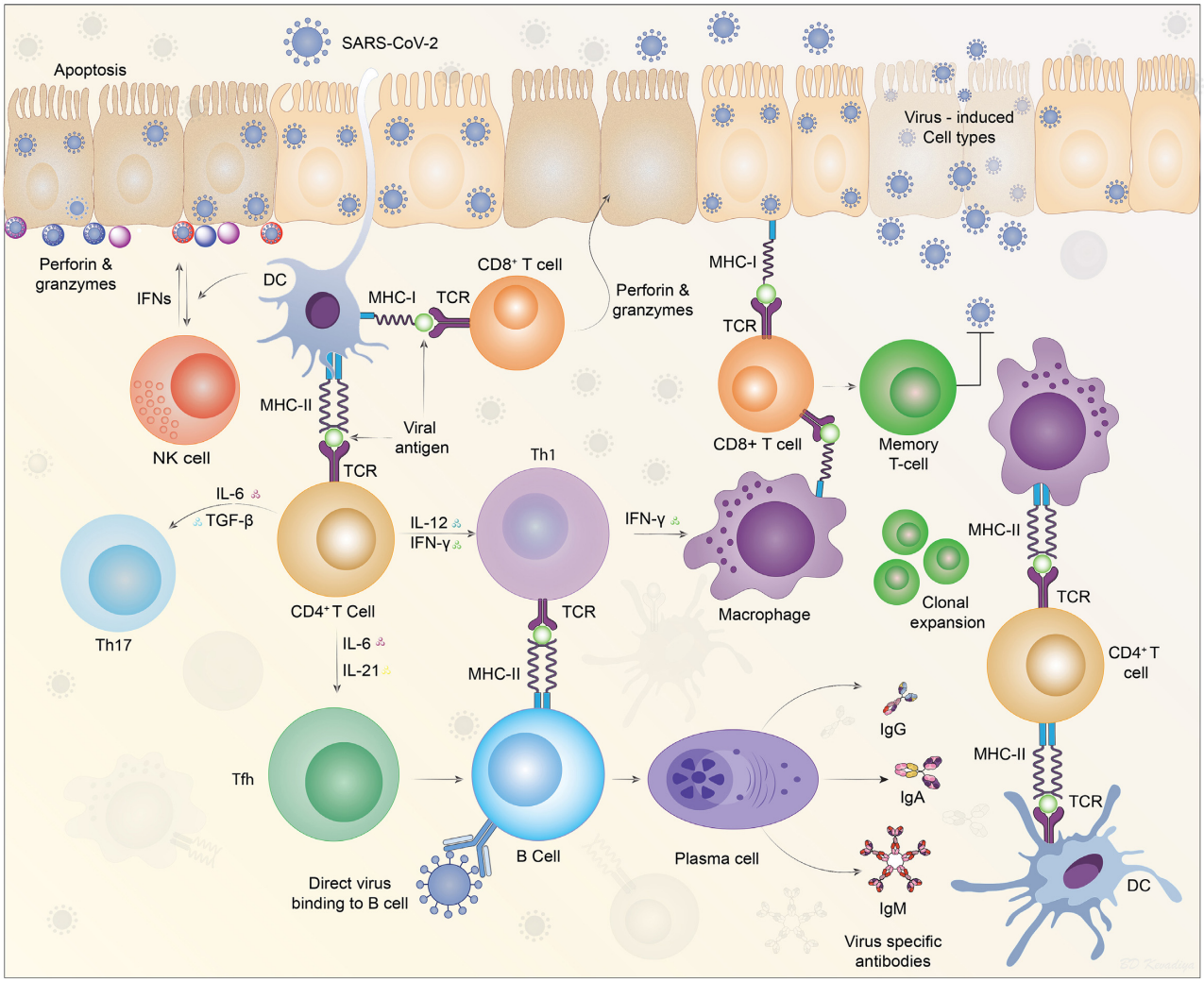

Figure 3. SARS-CoV-2 immunity. Following SARS-CoV-2 infection of epithelial cells, lysis and epithelial damage is one pathway. Another is in presentation of viral antigens from antigen presenting DCs or macrophages to CD8+T cells. The resultant mobilization of cytotoxic CD8+ T cells can recognize viral antigens on neighboring cells with the release of perforin and granzymes. This causes infected epithelial cells to undergo apoptosis. DCs can also present the viral antigens to CD4+ T cells and induce cell differentiation to memory Th1, Th17, and memory T follicular helper cells. These latter cells facilitate B cells differentiation into plasma cells promoting the production of IgM, IgA, and IgG isotype virus-specific antibodies. Tissue macrophages may also present viral antigens to mobilize CD4+T cell immune responses. (figure originally made in house by authors and concept adopted from these references (Jansen *et al.* 2019; Azkur *et al.* 2020).

receptors (Acharya *et al.* 2020; Walker *et al.* 2020). Anti-RBD monoclonal antibodies (mAbs) are commonly detected in tested patients. Although cross-reactivity to SARS-CoV S and N proteins as well as to MERS-CoV S protein were detected in plasma from COVID-19 patients, no cross-reactivity was found to the RBD from SARS-CoV or MERS-CoV. In addition, plasma from COVID-19 patients did not neutralize SARS-CoV or MERS-CoV (Liu *et al.* 2020).

The B cell response to infection serves not only to protect from the initial challenge, but also to offer extended immunity against reinfection. Following resolution of an infection, plasma cells formed during the acute and convalescent phases continue to secrete antibodies, thereby giving rise to serological memory. Memory B cells that are also formed during the primary infection constitute the second arm of B cell memory. Memory B cells can quickly respond to a reinfection by generating new high-affinity plasma cells. Long-term protection is achieved through the induction of long-lived plasma cells and memory B cells. There is renewed interest in understanding the lifespan of B cell memory responses to SARS-CoV-2 for the development of an effective vaccine. Protection from reinfection has direct medical and social consequences. In COVID-19 patients, evidence of near-universal seroconversion and the lack of substantial descriptions of reinfection point to a robust antibody response, which along with the T cell memory response, would offer protection from reinfection. Indeed, a case study of a single patient described induction of $CD38^{hi}CD27^{hi}$ antibody-secreting cells (Guo *et al.* 2020) concomitantly with an increase in circulating follicular T helper cells, whereas a scRNA-seq study of peripheral blood mononuclear cells from critically-ill and recently recovered individuals revealed a plasma cell population. In addition, IgG memory cells specific to the RBD have been identified in the blood of COVID-19 patients (Guo *et al.* 2020).

B lymphocytes are depleted in COVID-19 patients during advanced disease (Wang *et al.* 2020). Moreover, while those recovering from disease show reduced numbers of naïve B cells, those persons have increased circulating plasma- or antibody-producing cells in both polyclonal and monoclonal states (Wen *et al.* 2020). Increased extrafollicular B cell activation and numbers of antibody-producing cells result in high titers of SARS-CoV-2-specific antibodies (Woodruff *et al.* 2020). Despite specific B cell expansion and virus-specific antibodies,

disease progression occurs and is associated with high levels of inflammatory cytokines that result in organ failure and death. Interestingly, patients with X-linked agammaglobulinemia, which is characterized by a lack of mature B cells, tend to quickly recover from COVID-19 disease (Soresina *et al.* 2020; Tavakolpour *et al.* 2020). These observations support the notion that B cell responses may be less consequential in mounting early protective immune responses. Nonetheless, high titers of SARS-CoV-2-specific neutralizing IgG antibodies present in sera of convalescent individuals are protective (Ni *et al.* 2020). Correlations exist between increased antibody titers and viral loads as viral neutralization by antibody increases during the disease course (Huang *et al.* 2020), which correlates with the clonal expansion of B cells producing antibodies with higher neutralization capacities (Cox and Brokstad 2020). Viral neutralization parallels high antibody titers. B cells producing effective neutralizing antibodies show long-term immunity against SARS-CoV-2.

While convalescent plasma from 149 patients collected after 39 days (average) of infection showed low levels of neutralizing antibody, the ones that are produced were found to be highly potent with IC_{50} values in the nanomolar range. These data suggest that a vaccine with robust antibody production would be effective (Robbiani *et al.* 2020). When 20 vaccinated (Moderna, mRNA-1273 or Pfizer–BioNTech, BNT162b2) subjects were examined 20 days post second dose administration, they produced high levels of IgG and IgM and an equivalent level of memory B cell reflective of natural infection (Wang *et al.* 2021). A comprehensive study on a cohort of 87 patients showed that RBD-specific memory B cells remain unchanged after 6.2 months of infection, whereas the IgM and IgG antibody concentration decreased. The memory B cells in these patients after 6.2 months secreted antibodies that had increased potency, increased somatic hypermutation and resistance to RBD mutation (Gaebler *et al.* 2021). IgG specific to SARS-CoV-2 trimeric viral S protein were detectable in serum up to 60 days after symptom onset, but IgG titers began decreasing 8 weeks post-symptom onset. Long-term protection from reinfection may also be mediated by reactive memory B cells. A study that analyzed SARS-CoV protein-specific IgG memory cells at 2, 4, 6, and 8 months post-infection found that S-specific IgG memory B cells progressively decreased about 90% from 2 to 8 months after infection. A further retrospective study of 23 individuals found no evidence of circulating SARS-CoV- specific IgG memory B cells 6 years after infection. These results are in contrast to the memory T cell response, which was robustly detected based on induced IFN-γ production (Tang *et al.* 2011). The results suggest that immunity to SARS-CoV-2 may diminish following a primary infection, whereby further studies will be required to determine the degree of long-term protection.

Neutralizing antibodies (nAb) present in convalescent plasma generated in response to SARS-CoV-2 infection are considered to play an important role in viral clearance. Convalescent plasma infusion may also help in the modulation of immune responses, as will be discussed later in the *Immunotherapeutics* section. However, titers and kinetics of nAbs in infected patients are not well established, thereby precluding knowledge about the optimal time for plasma collection (Devarasetti *et al.* 2021).

IMMUNOTHERAPEUTICS

The COVID-19 disease outbreak worldwide urgently requires an effective treatment or preventive strategy, not only for attending to the clinical pathology of SARS-CoV-2, but also to curb the severe economic and social distress from the pandemic. Many therapeutic strategies have been employed so far, including mAbs against the virus, and cytokines, and their receptors, convalescent plasma therapy, and complement protein C5 (Yu *et al.* 2020). In addition, cytokine therapy, mesenchymal stem cell (MSC) therapy, and intravenous immunoglobulins have been investigated (AminJafari and Ghasemi 2020; Mansourabadi *et al.* 2020; Saghazadeh and Rezaei 2020). The reasons for adopting any of the immunotherapeutic strategies are due to the reports showing that acute inflammation, rather than the direct viral effects, cause life-threatening complications, consequent respiratory failure, and death (Saghazadeh and Rezaei 2020). Uncontrolled secretion of cytokines underlies the concept of cytokine storm, a term frequently interchanged with cytokine release syndrome (CRS). The overproduction of pro-inflammatory molecules, such as IFNs, interleukins, chemokines, CSFs, and TNFs, results in an inflammatory response whereby severe cases may result in organ failure. Cytokine storm is particularly important in the case of COVID-19 infection since it is responsible for airway obstruction and multi-organ failure (Wang *et al.* 2020). Clinical studies show high levels of IL-6, IL-1β, IL-8, IL-10, TNF, GM-CSF, IP-10, IL-17, MCP-1, MCP-3, MIP1-α, and IL-1RA in patients infected with SARS-CoV-2 (Ragab *et al.* 2020). If untreated, cytokine storm can result in ARDS and consequently progress to death. The deleterious effects from a cytokine storm underscores the importance of cytokines as therapeutic targets for the prevention of exacerbating infection. The following are strategies approved or are under clinical investigation for COVID-19 disease treatment.

Monoclonal antibodies

Neutralizing monoclonal antibodies may be able to prevent infection (Jiang *et al.* 2020). Neutralizing monoclonal antibodies showing high affinity and specificity to target antigen were employed to treat viral infections such as Ebola virus, cytomegalovirus, influenza virus, HIV-1, and respiratory syncytial virus. Neutralizing mAbs-based vaccination approaches in preclinical and clinical studies demonstrated that protection against SARS-CoV-2 and several investigations are underway by using antibody-based treatment options to combat COVID-19 disease (Garcia-Beltran *et al.* 2021). An ongoing trial in non-hospitalized COVID-19 patients with REGN-COV2, an antibody cocktail containing two SARS-CoV-2–neutralizing antibodies, was investigated. The aim of this trial was to reduce the viral load that may subsequently reduce the COVID-19-related complications and death. The neutralizing titers of REGN-COV2 were over 1000-fold higher when compared to titers achieved in the convalescent-phase of infection, which facilitated viral load reductions within two days (Weinreich *et al.* 2020; Zeng *et al.* 2020). This approach appears to be promising, as recent studies with other viruses (e.g., Ebola virus) using antibody cocktail demonstrated profound survival benefit for infected patients (Mulangu *et al.* 2019).

In another randomized, double-blind, placebo-controlled trial for BLAZE-2 (NCT04497987), Eli-Lily's nAb named bamlanivimab (LY-CoV555), was evaluated in the nursing home setting. The study evaluated LY-CoV555 in a relatively closed environment where SARS-CoV-2 was known to be circulating. Eligible participants were randomized to receive either 4200 mg bamlanivimab or placebo. The study results suggested that participants in LY-CoV555 treated group had 80% lower risk of contracting COVID-19 or manifesting symptomatic COVID-19 disease compared to the control group (McCully 2021). Also, the prevention data (primary and secondary analyses) on 965

participants (299 residents and 666 staff) who tested negative for the SARS-CoV-2 virus at baseline and also treatment data on 132 participants (41 residents and 91 staff) who tested positive for the virus at baseline. The primary outcomes measured was cumulative incidence of COVID-19 defined as the detection of SARS-CoV-2 by RT-PCR and mild or worse disease severity within 21 days of detection. The reported results were based on 8 weeks of follow-up for all participants and showed a reduction in symptomatic COVID-19 with bamlanivimab versus placebo. The odd ratio of 0.43 points to a reasonably large effect by clinical trial standards with an associated small p-value of <0.001. Such results provide convincing evidence of an effect. For the pre-specified subgroup of nursing home residents, there was also evidence of efficacy with a large effect (odd ratio of 0.20, p-value <0.001). However, for now bamlanivimab is authorized for emergency use by the U.S. FDA for the treatment of mild to moderate COVID-19 in high-risk patients (McCully 2021).

Altogether, the most promising immune-based approaches for disease rests in mAbs and in particular casirivimab and imdevimab. These are now approved by the U.S. FDA for mild to moderate COVID-19. It is administered after positive results for direct SARS-CoV-2 tests in patients at the highest risk for life threatening COVID-19 (Tantibanchachai 2020). These are individuals who are 65 years of age or older with comorbid conditions such as hypertension, diabetes, vascular and degenerative disorders. The administration of casirivimab and imdevimab together demonstrated reductions in a COVID-19-related hospitalization when compared to placebo. The safety and effectiveness of this antibody combination has been affirmed. Traditionally, cellular immunity is responsible for clearing an established viral infection, whereas humoral immune responses play a major role in preventing future infections (Tantibanchachai 2020).

Convalescent plasma therapy

Plasma from donors recovering from SARS-CoV-2 infection have antibodies against the SARS-CoV-2 virus and can help in modifying immune response. On August 23, 2020, the U.S. FDA provided EUA for plasma of patients convalescing from COVID-19, as no other approved effective treatments were available (FDA 2020). Beyond the virus-neutralizing activity of the plasma antiviral antibodies, the mechanisms by which convalescent plasma therapy (CPT) exerts its activities can include humoral and cellular immune responses (Nasser *et al.* 2013; Pelegrin, Naranjo-Gomez and Piechaczyk 2015). The latter include complement-dependent cytotoxicity and/or inactivation of viral particles and antibody-dependent cellular phagocytosis and cell-mediated cytotoxicity, to eliminate the viral antigen-expressing infected cells.

At the outset of the pandemic, an uncontrolled case series of 5 critically-ill patients with COVID-19 and ARDS were given CPT-based neutralizing antibody for treatment. These patients demonstrated an improvement in clinical status, which raised the possibility of using CPT to treat COVID-19 disease (Shen *et al.* 2020). Many studies followed to evaluate the safety and effectiveness of CPT for possible improved clinical outcomes (Duan *et al.* 2020; Maor *et al.* 2020). The study by Duan *et al.* enrolled 10 patients with severe COVID-19 disease that were dosed with 200 mL of convalescent plasma with high titer of neutralizing antibodies as an addition to the antiviral therapy and standard of care (SOC) (Duan *et al.* 2020). Clinical outcomes were favorable with an increase in neutralizing antibodies and lymphocyte counts, increased oxyhemoglobin saturation, reduction in viremia, varying degrees of absorption onto lung lesions, and delaying the onset of illness from 5–14 days (in non-CPT COVID-19 recipients) to 16.5 days (Duan *et al.* 2020). The open-labeled, randomized study enrolled 101 COVID-19 patients to evaluate the clinical improvements (in terms of hospital discharge time and reduction in disease severity based on a 6-point disease severity scale) within a 28-day period. Overall, there was no significant difference between CPT and non-CPT groups for clinical outcomes (Li *et al.* 2020). Unfortunately, interpretation was limited by the early study termination. Similarly, in another randomized placebo-controlled study, no significant differences in the primary clinical outcomes between the CPT-treated and the placebo group were observed (Simonovich *et al.* 2020).

Moreover, significant correlations between SARS-CoV-2-specific IgG antibody levels and neutralizing activity were reported. Recipients with higher IgG antibody levels showed improved clinical outcomes (Maor *et al.* 2020). These results underscore the importance of considering neutralizing antibody levels as critical factors for rescue therapy (Joyner *et al.* 2020; Marklund *et al.* 2020). A retrospective, propensity score–matched case–control study in 39 patients, admitted in Mount Sinai Hospital in New York City, assessed the effectiveness of CPT in the case of severe COVID-19 disease condition (Liu *et al.* 2020). This study revealed that the overall survival probability was greater in CPT recipients than that of controls. The comparison groups were adjusted for covariates such as duration of symptoms before admission, exposure to therapeutic anticoagulation, and broad-spectrum antibiotics, as well as additional covariates, including mechanical ventilation, corticosteroids, azithromycin, interventional antivirals, and IL-6 inhibitors for survival analyses. The proportion of patients with worsened oxygenation was reduced in the plasma group as compared to the matched controls; however, the study did not offer statistical significance between the groups. Based on the timeline of the patient recruitment, scant patient numbers, and lack of statistical power, the above-mentioned results only suggest that the plasma therapy is beneficial to the non-intubated patients, those with less than a week of symptoms, and those also receiving therapeutic anticoagulation.

Plasma transfusion limitations include allergic reactions in the recipients; unknown influence of the procoagulants present in the donor's plasma, especially for recipients with history of acute thrombotic conditions (Liu *et al.* 2020); transfusion-related lung injury and circulatory overload; and concerns for antibody-mediated enhancement of pro-inflammatory effects for disease (Dzik 2020; Xi 2020). Besides the physiological challenges, technical challenges are also abundant, such as large-scale plasma production with high antibody titers (in conformance with guidelines for convalescent plasma collection), screening for pathogens, and meticulous viral inactivation, all of which influence donor's plasma activities (Xi 2020). Addressing these challenges will influence the efficacy of passive antibody transfer and the statistical significance of the perceived clinical improvements. Therefore, larger randomized trials are needed to address the utility of plasma therapy and define which patient population that would best benefit from such therapy. As viable treatment options for COVID-19 disease are investigated, CPT may remain as the treatment of choice for critical patients.

Immune blockade

Although the exact mechanism of the COVID-19 related mortality is still unclear, overactive uncontrollable immune response

is probably the most accepted cause. Control of the immune response in patients can affect disease morbidity and mortality.

Pro-inflammatory cytokines

Elevated IL-6 levels have been observed in patients infected with SARS-CoV-2, whereby the levels can be correlated with the severity of infection. Considering the major role IL-6 plays in CRS and COVID-19 infection, such provides a rationale for developing therapeutic strategies for its blockade. IL-6 binds to the soluble as well as the transmembrane form of IL-6 receptor (IL-6R) and activates the JAK cascade upon interaction with gp-130. With that in mind, immunotherapeutic antibodies that bind to either IL-6 or IL-6R are under investigation. Tocilizumab (TCZ)-ACTEMRA® is a recombinant mAb against the IL-6 receptor therefore through competitive inhibition attenuates interaction of IL-6 with IL-6R. Its subsequent downstream proinflammatory cascade to offer limited immune cell proliferation and differentiation as well as reduced oxidative stress, which in turn, controls the onset and progression of the cytokine storm. Additionally, TCZ helps to mitigate chimeric antigen receptor (CAR) T cell-induced cytokine release syndrome (Le *et al.* 2018). Such mitigation suggests TCZ's therapeutic potential in severe COVID-19 pneumonia associated with cytokine storm (Le *et al.* 2018; Campochiaro *et al.* 2020). TCZ was found to be effective in suppressing proinflammatory cytokine systemic effects for reducing fever and disease severity in hospitalized patients (Kaye and Siegel 2020). A retrospective TCZ cohort study performed on COVID-19 intensive care unit patients across 13 hospitals was performed. The study found that patients treated with the TCZ demonstarated a significant decrease in hospital mortality (Hackensack Meridian Health Universal Observational COVID-19 database, NCT04347993) (Biran *et al.* 2020). However, other reports showed no significant impact on the clinical outcome with notable side effects that included elevated hepatic enzyme levels and increased risk for sepsis (Morena *et al.* 2020). The latter results were affirmed from a randomized clinical trial and showed no significant difference between TCZ-treated patients and controls. TCZ-treated hospitalized patients with COVID-19 pneumonia showed worsened clinical outcomes. It is speculated that the outcome variability may result from the timeliness of TCZ administration affecting TCZ-IL6R binding and would require higher doses to show any beneficial outcome. Administration of TCZ at the beginning of the cytokine storm may be the key to improve therapeutic outcomes (Campochiaro *et al.* 2020).

Sarilumab (SRL) is another such IL-6R antagonist that has potential to be used in SARS-CoV-2-associated CRS. SRL has demonstrated mixed results in terms of its clinical efficacy in COVID-19 patients. A study involving 15 patients admitted to a single institute showed improvements in respiratory parameters in 10 patients upon single- or double-dosing of 400 mg SRL, evidenced by increased ratio of arterial oxygen pressure to fractional inspired oxygen (PaO_2:FiO_2), and reduced lung consolidation areas and parenchymal ground glass opacity. Moreover, cytokine and C-reactive protein (CRP) levels were reduced in recovered patients compared to patients who died from SRL treatment arm. Alternatively, a separate study compared the therapeutic efficacy of SOC (lopinavir/ritonavir, hydroxychloroquine, azithromycin) in COVID-19 patients in presence and absence of SRL (Della-Torre *et al.* 2020). The SRL and SOC group showed no significant survival improvement compared to the SOC group; however, the median time until death was significantly longer in the SRL and SOC group than the SOC group. Both studies acknowledged that intervention with SRL at initial stages of SARS-CoV-2 infection (<17% consolidated lung as per the latter study) has potential to slow down CRS progression. Additional clinical studies are required to corroborate the findings. Unfortunately, some clinical trials were discontinued due to failure to meet the primary and secondary end points. Sanofi and Regeneron discontinued their trials (NCT04327388 and NCT04315298, respectively) as COVID-19 patients did not show any significant benefits in early investigations when SRL (Kevzara) was added to SOC therapy. Siltuximab (SLX) is also an IL-6 inhibitor. An observational cohort study was conducted that included 30 COVID-19 patients (Gritti *et al.* 2020) showed a lower 30-day mortality rate in those patients administered SLX and SOC.

IL-1β, a critical contributing cytokine to CRS, plays a key role during the early stages of cytokine cascade to activate macrophage and produce IL-6. IL-1-associated cytokine overproduction can be attenuated by immunotherapeutic antibodies that bind to either IL-1β or IL-1R. Anakinra is an IL-1R blocker. When administered at a high dose (5 mg/kg twice a day intravenously), anakinra reduced CRP levels and improved respiratory function in 21 out of 29 patients (72%) in combination with SOC compared to SOC alone group, while in standard treatment group (SOC alone) 8 out of 16 patients (50%) showed respiratory improvement after 21 days (Cavalli *et al.* 2020). A low-dose (100 mg twice a day subcutaneously) anakinra treatment in 7 patients along with SOC was discontinued because of worsened clinical status. This retrospective cohort study demonstrated 90% survival in the high-dose anakinra and SOC group compared to 56% in the SOC alone group. A separate study involved 12 patients in the anakinra and SOC group who received 300 mg/day anakinra for 5 days followed by tapering doses for the next 3 days (Cauchois *et al.* 2020). The control group comprised of 10 patients receiving SOC alone. No death was reported while significant reduction in oxygen requirement, CRP levels, and fever was observed in the anakinra and SOC group compared to SOC alone. In all, while the results were encouraging; randomized controlled trials will be required to validate the results.

Immunomodulation and immunopotentiation

GM-CSF is responsible for the activation of macrophages and neutrophils to secrete pro-inflammatory cytokines including IL-6, IL-1, IL-12, and TNF. GM-CSF blockade by therapeutics that bind to GM-CSF or GM-CSF receptor α (GM-CSF-Rα) can control COVID-19-associated CRS. COVID-19 pneumonia is induced by SARS-CoV-2 and can lead to the progression of ARDS. Apart from protective ventilation, fluid restriction, prone positioning, and extracorporeal membrane oxygenation, no specific therapeutic options exist. GM-CSF can promote differentiation and mobilization of different myeloid leukocyte subsets including neutrophils, tissue macrophages, and DCs or their circulating precursors. GM-CSF is crucial for alveolar epithelial repair following hyperoxia and inflammatory lung injury. The trial proved effective to prevent progression to ARDS in COVID-19 pneumonia patients by GM-CSF inhalation (Lang *et al.* 2020). In a second study, the IgG4 isoform of mavrilimumab, a GM-CSF-Rα blocker, was investigated and the results showed its efficacy in 13 COVID-19 patients compared with 26 COVID-19 patients receiving SOC (De Luca *et al.* 2020). All patients who received one dose of 6 mg/kg mavrilimumab showed early recovery (except for one patient) and no death was reported. In contrast, seven deaths were reported in the SOC group (Cremer *et al.* 2021). In

general, mavrilimumab reduced fever and serum CRP as well as improved lung opacification. Detailed, controlled clinical trials are warranted to establish broader treatment efficacy. Additionally, anti-GM-CSF antibodies such as gimsilumab, lenzilumab, and TJ003234 are being tested in COVID-19 patients (Bonaventura *et al.* 2020). GM-CSF is known to have both pro- and anti-inflammatory responses and can transform effector T cells into regulatory cells (Bhattacharya *et al.* 2015). It is also known to facilitate pathogen clearance. Thus, boosting GM-CSF appears as a more efficacious strategy over its blockade for treatment of life-threatening ARDS morbidities (Matthay *et al.* 2019).

Chemokine receptor

The CCR5 receptor is present on the immune cell membranes and is responsible for chemokine-induced tissue migration of immune cells. CCR5 blockade is an effective way of controlling the cytokine storm. Leronlimab, a CCR5 blocking antibody, reduced IL-6, restored CD4:CD8 T cell ratio, and reduced viremia in 10 terminally-ill patients (Patterson *et al.* 2021).

Signaling pathway

Inhibition of JAK, mainly JAK-1 and -2, prevents phosphorylation of STAT3 protein, hence blocking the JAK-STAT pathway that responsible for the secretion of pro-inflammatory cytokines such as IL-10 and IL-6. Baricitinib is a reversible JAK inhibitor and was given to 20 COVID-19 patients at a dose of 4 mg twice daily for two days followed by 4 mg daily for the remaining 7 days (halved doses for patients more than 75 years of age) (Bronte *et al.* 2020). Viral S protein-specific IgG levels were significantly increased, and in the one recorded patient death said patient did not present serum IgG. Reduced levels of CRP, IL-6, TNF-α, and IL-1β were observed and oxygen demand reduced faster compared to the control group. However, no clinically significant outcomes in terms of fever reduction, disease duration, or ARDS incidence were experienced. Hence, after acknowledging the study limitations which included short follow-up and data lapses, the efficacy of baricitinib for COVID-19 requires a complete randomized clinical trial. Beneficial effects were observed in a separate study when baricitinib was combined with hydroxychloroquine; however, the use of baricitinib in COVID-19 patients needs further investigation considering no control group was included in the study (Titanji *et al.* 2021). Ruxolitinib is also a JAK/STAT blocker that is under evaluation for treating COVID-19 disease.

Apart from the above strategies, options such as MSC transplantation and CPT approaches are promising avenues in COVID-19-associated CRS. For instance, MSC transplantation showed beneficial outcomes in 7 patients as evidenced by reduced cytokines, the disappearance of $CXCR3^+CD4^+$ T cells, $CXCR3^+CD8^+$ T cells, $CXCR3^+$ NK cells, and the increase in the population of $CD14^+CD11c^+CD11b^{mid}$ regulatory DC cells (Leng *et al.* 2020). The transfusion with 200 mL CPT rescued 10 severe patients in a study that showed vanquished viremia with maintained or increased levels of neutralizing antibodies and lung lesion absorption (Duan *et al.* 2020). Another study that involved seriously-ill patients showed resolution of ARDS in 4 out of 5 patients, reduced fever, and improved PaO_2:FiO_2 (Campochiaro *et al.* 2020; Shen *et al.* 2020). Overall, different approaches are being explored to control the cytokine storm in SARS-CoV-2-infected patients, as will be discussed further in the next sections. It is essential to establish the safety and efficacy in larger trials.

Cell-based therapies

The systemic inflammatory response that is referred to as the cytokine storm results in widespread lung damage, thereby contributing to the pathological outcomes in SARS-CoV-2 affected patients. Thus, prevention or attenuation of this cytokine storm can serve to mitigate ARDS.

Mesenchymal stem cells

In the past, mesenchymal stem cells (MSCs) were demonstrated to possess immunoregulatory functions by affecting the B cells, T cells, monocytes, macrophages, and DCs (Han *et al.* 2019; Weiss and Dahlke 2019). MSCs have immunosuppressive capacities that provide new insights into the treatment of immune-mediated diseases (Wang Yuan and Xie 2018). The registry on the National Institutes of Health (NIH) Clinical Trial Databank (https://clinicaltrials.gov/) shows over 100 clinical trials linked to the immunomodulatory effects of MSCs, which include HIV studies that utilize the immune control functions of MSCs (Thanunchai, Hongeng and Thitithanyanont 2015). The immunoregulatory and immunosuppressive capacities of MSCs take advantage of affecting COVID-19 patient outcomes (Raza and Khan 2020). For example, preliminary results from a phase I study demonstrated the proof-of-concept that adipose tissue-derived MSCs, when administered to critically-ill patients with COVID-19 pneumonia, changed their inflammatory and immune status. These status shifts suggest potential benefits of MSCs in patients with mechanical ventilation. Similar studies were also conducted using MSCs from different sources such as human umbilical cord. Overall, of 62 current clinical trials, the outcomes evaluated include the safety of the respective sources of MSCs in treatment of ARDS; the proportion of patients requiring ventilator support that can breathe without assistance, as well as oxygenation and respiratory parameter measurements; treatment effects on circulating blood inflammatory cytokines and immune cells; measurements of markers for infection (e.g. CRP) and organ function (e.g. creatine kinase and alanine aminotransferase); improvement of clinical symptoms (fever and respiratory); and mortality rates (Durand, Mallea and Zubair 2020).

Moreover, extracellular vesicles derived from MSCs (MSC-EVs) were found to promote therapeutic activities comparable to those exhibited by the source MSCs (Machhi *et al.* 2021). Despite only a few studies that directly compare MSCs to MSC-EVs, the overlapping biological effects suggest MSCs to be merely the vehicle and that MSC-EVs have a greater likelihood of impacting areas affected by inflammation (Fujita *et al.* 2018). There are very few studies that investigated the immunoregulatory capacities of MSC-EVs (Gowen *et al.* 2020). There is only one clinical trial (not yet recruiting) to evaluate the safety and efficacy of intravenous administration of bone marrow-derived EVs versus placebo as treatment for ARDS in patients with severe COVID-19 (NCT04493242). Extensive studies need to be performed to determine the best option between MSCs and MSC-EVs for mitigating the inflammatory status of COVID-19 patients.

Regulatory T cells

Regulatory T cells (Tregs) are important for immune homeostasis. Tregs inhibit the activation of both innate and adaptive immune cells by utilizing inhibitory surface molecules [cytotoxic T lymphocyte antigen-4 (CTLA-4) and lymphocyte-activation gene-3 (LAG-3)] and by secretion of immunosuppressive cytokines IL-10, TGF-β, and IL-35 for immune regulation

(Wing, Tanaka and Sakaguchi 2019). A report showed that the level of peripheral Tregs is markedly reduced in severely-ill COVID-19 patients, but such takeaways are muddled by the perturbations of certain Treg phenotypes (Romano *et al.* 2019; Diao *et al.* 2020; Galván-Peña *et al.* 2020). Bronchoalveolar lavage-derived $CD4^+$ T cell transcriptomic data suggests that IL-2 transcripts are reduced in severe cases and such reduction in IL-2 would lead to enhanced apoptosis of Tregs. It is hypothesized that this reduction in Treg levels could be one of the reasons for the hyperactivated immune system and lung damage in severe COVID-19 patients (Stephen-Victor *et al.* 2020). Recently, the *ex vivo* transfer of polyclonal Tregs has been recently used to treat autoimmune and inflammatory diseases, such as type 1 diabetes mellitus (Bluestone *et al.* 2015). The study aimed to show that allogeneic transfer of Tregs would be beneficial to replace and reverse the autoimmune condition and subsequent damage of pancreatic beta cells in diabetic patients. A disadvantage of peripheral blood-derived polyclonal Tregs therapy is the length of time it requires to generate viable clinical-grade Tregs, which is unsuitable for COVID-19 patients who require instant therapy to prevent comorbidity and mortality. Therefore, use of allogeneic human leukocyte antigen-matched umbilical cord-derived Tregs (instead of autologous polyclonal Tregs) are under exploration for inflammatory conditions (ongoing clinical trials registered at NCT02932826 and NCT03011021) (Romano *et al.* 2019; Stephen-Victor *et al.* 2020). Two case reports of COVID-19 patients with ARDS who were treated twice or thrice with allogeneic, off-the-shelf, cord blood-derived, *ex vivo*, expanded Tregs (1×10^8 cells per dose) showed rapid decline in various inflammatory mediators including IL-6, TNF-α, IFN-γ, IL-8 and IL-12 (Gladstone *et al.* 2020). For certain, there is a paucity of studies using Tregs, but more extensive investigations will aid in evaluation of the scalability and benefits of using Tregs as part of immunotherapy.

NK cell therapies

NK cells are innate immune responders for viral clearance and immunomodulation (Market *et al.* 2020). Despite the data linking inflammation with COVID-19 disease severity and the potential of NK cells in mediating immunopathology, there is a dearth of studies to demonstrate the contributions of NK cells to SARS-CoV-2 clearance. Some clinical trials are ongoing to determine the safety and efficacy of the NK cells, such as those investigating (i) NK cells derived from human placental $CD34^+$ cells (CYNK-001) for SARS-CoV-2 clearance and clinical improvements (NCT04365101); (ii) IL-15 super agonist and GM-CSF neutralizing single chain variable fragment for clearance of SARS-CoV-2 virus particles and their infected cells (NCT04324996); and (iii) NK cells in combination with standard therapy for pneumonia patients infected with SARS-CoV-2 (NCT04280224). More extensive investigations are required to evaluate the clinical benefits of NK cells and other cell-based therapies (Pashaei and Rezaei 2020).

Immune modulation therapy

Type 1 interferons are crucial for antiviral immunity. IFNs are a group of cytokines that consists of ubiquitous α, β and γ subtypes (with many isoforms). They are primarily secreted by the plasmacytoid DCs from virus-derived components, which interact with pattern recognition receptors. Recognition of IFNs by its receptors, which are found on plasma membrane of most cell types, induce a signaling cascade that leads to the induction of IFN-stimulated genes. IFN-stimulated genes are involved in signaling and immunomodulation, which can interfere with viral replication. IFN-stimulated genes spread by upregulating PRPs and reducing membrane fluidity to slow down viral egress (Totura and Baric 2012). A robust IFN1 response contributes to defense against viral infection by potentiating the recruitment and activation of various immune cells. On the other hand, uncontrolled or prolonged IFN1 production can produce inflammatory comorbid disease (Uggenti, Lepelley and Crow 2019).

SARS-CoV-2 Mutant virus

Genetic variation in viruses over time lead to the emergence of new variants with different characteristics. The variants may present concerns such that they spread easier, cause more severe disease, or may escape the body's immune response. To further complicate matters, the variants may evade detection by specific diagnostic tests and decreased susceptibility to medical therapies. In the event that significant mutations accumulate in in the viral S protein, the viruses may be able to evade immunity induced by vaccines or by natural infection (CDC 2021). Several new variants emerged globally, and scientists are attempting to learn about the virologic, epidemiologic, and clinical characteristics of these variants to better understand their transmission and how the mutation may affect the effectiveness of currently authorized vaccines. A few of the emerging variants are discussed here. The variant, B.1.1.7 lineage (a.k.a. 20I/501Y.V1 Variant of Concern (VOC) 202012/01), was detected first in the United Kingdom in September 2020 (Kirby 2021). Sequencing revealed 14 genetic mutations, 8 of which occurred in parts of the genome that encode the viral S protein and which impairs detection of the S gene (Andrew Rambaut *et al.* 2020). These mutations seem to have contributed to phenotypic change of the viral S protein, resulting in changes to the cell binding mechanism, with the potential for increased infectivity (Davies *et al.* 2021; du Plessis *et al.* 2021).

The current surge of COVID-19 cases across the United Kingdom now includes another highly transmissible SARS-CoV-2 variant (B.1.351/501Y.V2). This South African 501Y.V2 variant is characterized by three mutations in the SARS-CoV-2 viral S protein, one of which (N501Y mutation) is also present in the UK VUI-202,012/01 variant (Tang, Tambyah and Hui 2021). Consequences of the specific mutations in P.1 lineage (a.k.a. 20J/501Y.V3) at the population level have not been extensively studied, despite the detection of the 501Y.V2 variant, colloquially known as South African COVID-19 variant, in 26 countries. Currently, the 501Y.V2 variant is suggested to be a more highly transmissible strain due to the speed at which it became predominant in this South African population (Davies *et al.* 2021; Tang*et al.*2021). There is currently no data on the impact of the target S gene mutation on assay performance. However, there is no indication of increased severity of illness, though research is still ongoing in both the UK and South Africa to further characterize the phenotype of this variant. There are many other variants of concern that have emerged besides the above-mentioned ones that prevail, the updated list of which can be found in 'Coronavirus antiviral & resistance database' by Stanford University (Database 2021; FDA 2021).

Due to the emergence of multiple variants, FDA recommends clinical laboratory staff and health care providers who use SARS-CoV-2 tests to be aware of the fact that false negative test results may arise due to the prevalence of mutant variants. Therefore, clinical observations, patient history, and epidemiological

information must also be considered to evaluate test results or employ alternative diagnostic tests for re-confirmation (Tegally *et al.* 2020) (Fig. 4).

VACCINES

Three vaccines have been administered in the US under FDA emergency use authorization (EUA): BNT162b2 from Pfizer-BioNTech, mRNA-1273 from Moderna, and Ad26.COV.S from Johnson&Johnson/Janssen (FDA 2021). The EUAs were granted December 11 2020, December 18 2020, and February 27 2021, respectively. None of these currently administered vaccines have been fully FDA approved, but they are in the process of finishing phase III clinical trials. As April 15 2021 over 78 million people (over 23% of the total population) in the US have been fully vaccinated and over 34 millions of that total are 65 years and older (US CDC Data Tracker). As of the same date, over 198 million doses have been administered. Only about 7 million of those doses have been Johnson&Johnson while the remaining doses have been Moderna and Pfizer-BioNTech. On April 13 2021, the CDC and FDA jointly recommended pausing the administration of the Johnson&Johnson vaccine after which they conducted an Advisory Committee of Immunization Practices meeting over the vaccine (CDC 2021). As of April 10 2021 the US 7-day moving average for administered doses was approximately 3.0 million doses a day with single day peak at 4.1 million doses administered (US CDC Data Tracker). Comparatively, Canada has four approved vaccines. The same three as the US plus the AstraZeneca ChAdOx1-S, but as of March 27 2021, no one has received the Johnson&Johnson vaccine (Government of Canada). As of April 15 2021 per the government, just over 13% of the population has received one dose, while less than 2% have received two doses. As of April 8 2021, over 10 million doses have been administered in Mexico with 5 different vaccines approved but only 2 are currently being administered, Pfizer-BioNTech and China's CasSino Ad5-nCoV (Mexican Secretary of Health). Beyond just the major countries of North America, vaccine distribution has been begun on other major continents and highly populous nations like India and China. In the European Union as of April 3 2021, approximately 82 million doses across Europe have been administered as reported by 30 countries. Of the eligible population, a median of 6.7% has been fully vaccinated (range: 1.6-12.7%) as reported by 29 countries (European Centre for Disease Prevention and Control). In China 167.34 million vaccine doses have been administered across their nation, as of April 11 2021 (National Health Commission of the People's Republic of China). In India per the Indian Ministry of Health, just over 100 million total doses have been administered, as of April 13 2021 (Indian Ministry of Health). Those numbers represent the scale at which some of the larger nations and unions are operating at, but of course vaccines are being administered across the globe to nations of varying sizes.

Recombinant proteins

Viral vector-based vaccines allow intracellular antigen expression and induce cytotoxic T lymphocyte responses. Human adenovirus serotype 5 (Ad5) is a widely used gene delivery vector because it can be produced in high titers, has high transduction efficiency, and high transgene expression. A randomized, double-blind, placebo-controlled, phase II trial of the Ad5-vectored vaccine was developed by the Beijing Institute of Biotechnology (Beijing, China) and CanSino Biologics (Zhu *et al.* 2020). This study evaluated safety, efficacy and reactogenicity in volunteers between 18 and 85 years. The replication-defective Ad5 vector expressing the full-length gene encoding for the spike S protein was based on Wuhan-Hu-1 (GenBank accession number YP009724390). A single injection of vaccine with doses of 5×10^{10} to 1×10^{11} viral particles per mL or placebo, was given to participants intramuscularly and then the participants were observed for 14 and 28 days. The aims of the study were to find the dose, evaluate safety (number of adverse events), and determine immunogenicity (geometric mean titers of receptor binding domain- specific antibody responses and neutralizing antibody responses against live-virus or pseudovirus post-vaccination). The phase II study revealed that a single injection of 5×10^{10} viral particles has an adequate safety profile and elicits significant specific immune response, thereby making it eligible for acute protective vaccination (Zhu *et al.* 2020). Insight into further clinical trials of this vaccine is limited due to the collaboration in testing between the China's People's Liberation Army and the vaccine developers (Lewis 2020). This vaccine is currently approved for use in China, Chile, Mexico and Pakistan.

Ad5 induces potent immune responses. The presence of pre-existing immunity to the virus can inactivate subsequent immune responses and interfere with transgene expression (Tan *et al.* 2013). To overcome this issue and improve its clinical use, adenoviral vectors of non-human origin are developed, such as the chimpanzee virus-derived vector ChAd63 (O'Hara *et al.* 2012). The University of Oxford had designed the ChAdOx1 nCoV-19 vaccine (AZD1222) consisting of the replication-deficient simian adenovirus vector ChAdOx1. It comprises the full-length structural surface glycoprotein (spike S protein) of SARS-CoV-2, along with a tissue plasminogen activator leader sequence (Folegatti *et al.* 2020). ChAdOx1 nCoV-19 vaccine expresses a codon-augmented coding sequence for the spike S protein (GenBank accession number MN908947). A phase I/II clinical trial (ISRCTN15281137, NCT04324606) with the chimpanzee adenovirus-vectored vaccine (ChAdOx1 nCoV-19) was compared with a meningococcal conjugate vaccine (MenACWY) to maintain blinding to local and or systemic reactions to the viral vector (Folegatti *et al.* 2020). Healthy participants (age 18–55 years) were given ChAdOx1 nCoV-19 at a dose of 5×10^{10} viral particles by intramuscular injection. The clinical results showed that a single dose of ChAdOx1 nCoV-19 induces both cellular and humoral response in healthy volunteers with no severe adverse events related to ChAdOx1 nCoV-19. In the ChAdOx1 nCoV-19 group, spike S-specific T cell responses peaked on day 14. Antibodies against SARS-CoV-2 spike S protein rose by day 28 and were boosted following a second dose at day 56 in the 10 participants who received a booster dose. The preliminary results of this first-in-human medical trial backed clinical development cycle into continuing phase II/III trials. This vaccine, ChAdOx1 nCoV-19/AZD1222, is currently being mass-produced by the Serum Institute of India for global distribution (Voysey *et al.* 2021). The vaccine was found safe and less than 1% of vaccine recipients experienced serious adverse events. The data across four studies enrolling 24,422 participants established the vaccine efficacy of 66.7% at the endpoint analysis done greater than 2 weeks after the second dose. Even a single dose exhibited 76% efficacy. Exploratory modeling analysis indicated the efficacy of 81.3% after the second dose when the prime-boost interval was greater than 12 weeks. These properties coupled with less restrictive storage requirements are beneficial for early community-wide immunization efforts, especially in developing countries. However, ChAdOx1 nCoV-19 two-dose regimens has shown minimal protection against the B.1.351 COVID-19 variant in phase I/II double-blind clinical trials (NCT04444674) (Madhi

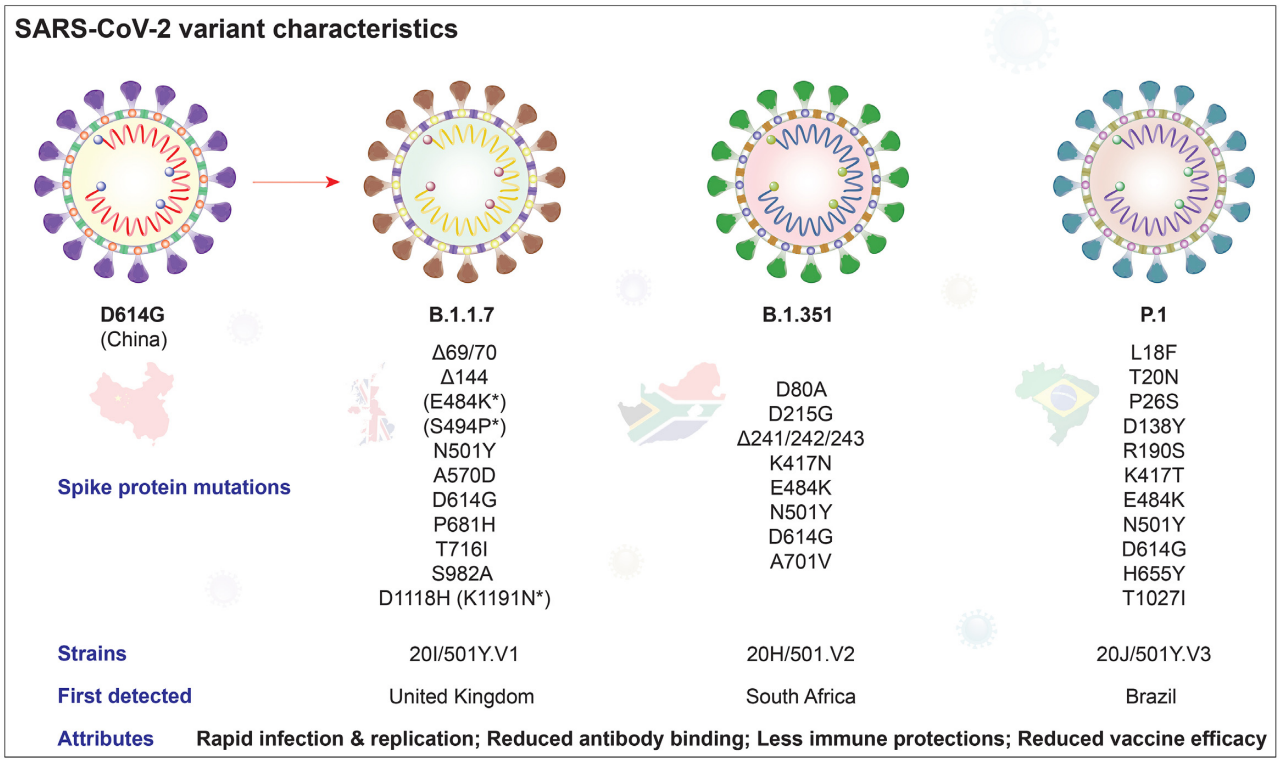

Figure 4. Characteristics of novel variants of SARS-CoV-2. Genetic mutations in SARS-CoV-2 (origin: Wuhan City, China) are generating novel viral variants, such as the three popular strains - B.1.1.7 United Kingdom (UK), B.1.351 South Africa (SA), and P.1 Brazil. The mutation can change many attributes including transmission rates and severity of disease. The reduction in neutralization capacity due to mutation could affect the current vaccination strategy (CDC 2021). The B.1.351 and P.1 variants (also known as 501Y.V2 and 501Y.V3) that have emerged in South Africa and Brazil, respectively, has additional mutations in the RBD at positions E484 and K417. Viral variants with the triple combination of N501Y, E484K and K417N/T have significantly reduced susceptibility to vaccine-induced and convalescent sera (Cele *et al.* 2021; Gupta 2021). (*) = detected in some but not all sequences. Introduction of the mutation that encodes the E484K substitution in the B.1.1.7 led to a more-substantial loss of neutralizing activity by vaccine-elicited antibodies (Collier *et al.* 2021; Wise 2021) (figure originally made in house by authors).

et al. 2021). In further on-going clinical trials in the UK and overseas, they have recruited high-risk people like healthcare workers and older age groups with comorbidities to assess efficacy, safety and immunogenicity of ChAdOx1 nCoV-19 vaccine. Based on the positive results from phase III clinical trial in US that is evaluating vaccine effectiveness in diverse populations, ChAdOx1 nCoV-19 has just begun phase IV trials (NCT04760132).

Another, adeno-based viral vector vaccine Gam-COVID-vac (Sputnik V) was designed by Gamaleya Research Institute of Epidemiology and Microbiology and the Health Ministry of the Russian Federation. It is currently under phase III clinical trials (NCT04530396) (Logunov *et al.* 2021). Gam-COVID-Vac is two viral vector vaccines, which will enter human cells and stimulate an immune response. Gam-COVID-vac is combination of rAd type 26 (rAd26) and rAd type 5 (rAd5), each carrying a full-length spike protein S-antigen (rAd26-S and rAd5-S). Both are separately administered intramuscularly with 21-day interval. Phase I/II trials were completed in August 2020, and the safety profile and cellular immune responses among healthy participants. Russia's President Vladimir Putin surprised the world by approving its first domestically developed COVID-19 vaccine before phase III clinical trials had begun (Baraniuk 2021). Interim analysis of phase III trials displayed 91.6% efficacy and good tolerability profile among participants 18 years and older (Logunov *et al.* 2021). There is no other vaccine other than Sputnik V publicly available in Russia. The Russian government has, however, approved two other Russian developed vaccines for emergency use; (i) EpiVacCorona, produced by the Vector Institute in Novosibirsk, and (ii) CoviVac, from the Chumakov Centre in St Petersburg Clinical trial (NCT04527575)(Vaccinations 04/11/2021, Reuters 2021). Yet another phase I-IIa trial vaccine, Ad26.COV2.S developed by Janssen Pharmaceutical carries replication-incompetent adeno serotype 26 encoding SAR-CoV-2 spike protein (Sadoff *et al.* 2021). Ad26 vaccine has also been used for EBOLA and was approved by European medicine agency for Zika virus and HIV. The randomized, double-blind phase III trials of Ad26.COV2.S were found to be safe and immunogenic in young and adults and showed 66% efficacy (NCT04505722).

Other promising recombinant subunit candidate include two-component spike nanoparticles, which are in preclinical studies (Brouwer *et al.* 2021). These efficiently scalable, self-assembling protein nanoparticle systems allows high-density antigen (prefusion S protein) presentation to facilitate greater immunological responses. Studies in rodents and macaques demonstrated improved antibody titers and reduction in viral loads (in both upper and lower respiratory tracts) in vaccinated animals. Potent nAbs from COVID-19 patients have multiple targets of vulnerability (Brouwer *et al.* 2020). Platforms as such offer promises of improving immunological responses against SARS-CoV-2 as well as provide options for alternative route of administration, for instance nasal route, to target the mucosal entry point of SARS-CoV-2.

RNA

RNA vaccines that encode viral antigens are demonstrated to possess valid safety profiles and immunogenicity in several clinical trials. Multiple vaccines have been shown to protect against SARS-CoV-2 and are important to provide wide-spread immunity. Some of the vaccine candidates described below are being used globally to limit the spread of COVID-19 infection with promising outcomes.

BNT162b2 (BioNTech and Pfizer mRNA) (Polack *et al.* 2020)

The mRNA vaccine developed by BioNTech, and Pfizer encodes the viral spike S antigen and has demonstrated positive safety profiles. A controlled, observer-blinded dose escalation phase I study (NCT04368728) on healthy volunteers investigated a 2-dose schedule (separated by 21 days) at different dose levels of the vaccine candidate, BNT162b1 and BNT162b2, also in different age groups (Mulligan *et al.* 2020). BNT162b1 is a lipid nanoparticle-formulated, nucleoside-modified, mRNA vaccine that encodes trimerized SARS-CoV-2 spike S protein RBD. The preliminary results of RBD-binding IgG concentrations, SARS-CoV-2 neutralizing titers, and low systemic adverse events, support the success of mRNA vaccines. Another study (NCT04380701) with BNT162b1 shows robust RBD-specific antibody production, favorable responses of T cells, and cytokines induction by the BNT162b1 mRNA vaccine. These findings demonstrated the beneficial immunogenic mechanisms by which this mRNA vaccine can protect against SARS-CoV-2 infection. BNT162b2 mRNA vaccine makes use of cellular protein synthesis machinery to produce spike proteins that are presented on the surface of immune cells. Spike proteins are viral antigens that generate an immune response. The mRNA encoding membrane-anchored full-length spike protein of SARS-CoV-2 stabilized in lipid nanoparticle (LNP) formulation was tested for the safety and efficacy of its 21 days interval dual dose regimen. A total of 43,548 participants above 16 years of age were randomly assigned (1:1) to either treatment (30 μg per dose) or placebo groups in a multinational, observer-blind efficacy trial with the endpoint of median 2 months after the second intramuscular (IM) dose. The vaccine efficacy was seen to be 95% after the second dose (also 52% after the first dose) in participants with or without prior SARS-CoV-2 infection. More importantly, a similar extent of efficacy was seen in participants with coexisting hypertension. Local reactions were resolved within 2 days. Fatigue and headache were common, with serious adverse events similar to placebo and hence the vaccine was deemed safe for administration (Polack *et al.* 2020).

Results from phase III clinical trial consisting of 46,307 trial participants build upon and confirm previously released data and demonstrate strong protection against COVID-19 through six months post-second dose (FDA 2021). Of the 927 confirmed symptomatic cases of COVID-19 in the trial, 850 and 77 cases of COVID-19 were in the placebo and BNT162b2 group, respectively, corresponding to a vaccine efficacy of 91.3% (95% confidence interval [CI, 89.0, 93.2]). Consistent across age, gender, race, and ethnicity demographics, it was observed that BNT162b2 vaccine was 100% efficacious against the severe COVID-19 disease by the CDC definition (95% CI, [88.0,100.0]) versus 95.3% efficacy by the FDA definition (95% CI, [71.0, 99.9]). From the 697 cases of COVID-19 that were observed in the United States, 647 cases were observed in the placebo group versus 50 in the vaccine group, indicating vaccine efficacy of 92.6% (95% CI, [90.1, 94.5]). In South Africa, only 9 out of 800 enrolled participants suffered from COVID-19, all in the placebo group, rendering a vaccine efficacy of 100% in this trial (95% CI, [53.5, 100.0]). 6 of the 9 positive cases were the B.1.351 variant, which is prevalent in South Africa. The high vaccine efficacy observed through and up to six months following a second dose and against the variant prevalent in South Africa provides further confidence in the BNT162b vaccine's overall effectiveness. Published data from late 2020 revealed the BNT162b2 vaccine to be 95% effective in placebo-controlled, multinational, observer blinded trial which was a major steppingstone towards getting vaccine approval across multiple countries (Mahase 2020; Polack *et al.* 2020; Chagla 2021). The publication of these results was soon followed by the US granting the BNT162b2 vaccine from Pfizer-BioNTech the first emergency use authorization for a COVID-19 vaccine (FDA 2021). Most recently, published data on vaccine efficacy against SARS-CoV-2 in Qatar (Abu-Raddad, Chemaitelly and Butt 2021) (predominant variants being either B.1.351 or B.1.1.7) showed approximately 20 percentage points lower effectiveness than in clinical trials (>90%) and in real-world conditions in Israel (Dagan *et al.* 2021) and the USA (Thompson *et al.* 2021). The company continues to evaluate data from their landmark Phase 3 clinical trial (Polack *et al.* 2020), while also initiating new trials in special populations, such as pregnant women (Shimabukuro *et al.* 2021) and children under 12 (Frenck *et al.* 2021).

mRNA-1273 (Moderna mRNA platform) (Baden *et al.* 2021).

Beyond the Pfizer-BioNTech vaccine, mRNA-1273 is a second effective vaccine that also uses an mRNA-based platform to encode for spike protein. This vaccine was developed by Moderna in cooperation with the National Institutes of Allergy and Infectious Diseases. It is a lipid nanoparticle that encapsulates an mRNA vaccine that encodes the spike glycoprotein of SARS-CoV-2. It is administered at a 28-day interval for a dual dose regimen. A total of 30,420 participants above 18 years of age were randomly assigned (1:1) to either treatment (100 μg per dose) or placebo groups in a randomized, double-blind efficacy trial with the endpoint of median 2 months. The efficacy was 94.1% in preventing symptomatic infection in uninfected recipients with no severe COVID-19 disease observed in the treatment group. Efficacy of 93.6% was observed in seropositive patients at baseline. Fatigue and headache were common adverse events. More solicited systemic adverse events were observed in the treatment group after the second dose (79.4%) compared to placebo (36.5%); however, it lasted only up to 3 days after injection. Overall, the vaccine was deemed safe.

Despite the reassuring vaccine regimen and platform, a recent study has revealed a potential risk of illness after successful vaccination and subsequent infection with variant virus (e.g. with mutations - E484K, T95I, del142–144, and D614G) (Hacisuleyman *et al.* 2021) even after the second, booster dose. Also, other studies have shown the effects of mutations to reduce the sensitivity to immune sera (Collier *et al.* 2021) and loss of neutralizing activity by vaccine-elicited antibodies (Collier *et al.* 2021; Gupta 2021). Such observations underscore the importance of the ongoing race between immunization and the natural selection of potential viral escape mutants. These observations definitely do not undermine the importance of the urgent efforts being taken at the federal and state levels to vaccinate the USA population. Rather it highlights the importance of instituting mitigation strategies, including serial testing of asymptomatic

individuals, open publication and analysis of vaccination and infection databases (such as those accruing data in New York City), and rapid sequencing of SARS-CoV-2 mRNA obtained from a variety of high-risk individuals (Hacisuleyman *et al*. 2021).

DNA

DNA-based vaccines are comprised of plasmid(s) that contain the DNA sequence encoding the antigen(s) against which an immune response is sought. This type of vaccine relies on *in situ* production of the target antigen, thereby conferring the advantage of stimulating both humoral and adaptive immune responses (Hobernik and Bros 2018). However, the level of these responses were often insufficient to elicit significant clinical benefits. There are currently no approved DNA-based vaccines available for human use.

Inovio Pharmaceuticals, USA, is a biotechnology company focused on developing DNA based approaches to treat HPV, cancer, and infectious diseases. Inovio's DNA vaccine INO-4700 against MERS-CoV, a related coronavirus, has moved to a phase II trial. However, for SARS-CoV-2 infections, Inovio has initiated a randomized phase II/III placebo-controlled study (NCT04642638) in collaboration with Coalition for Epidemic Preparedness Innovations to evaluate the safety, tolerability, and immunological profiles of a DNA vaccine administered by intradermal injection followed by electroporation using a CELLECTRA® 2000 device. INO-4800 contains the plasmid pGX9501, which encodes for the full length of the spike S protein of SARS-CoV-2. In preclinical studies, authors demonstrated virus-neutralizing activities using three separate neutralization assays: (i) an assay using live SARS-CoV-2 viruses; (ii) an assay using a pseudo-virus, where another virus displays the SARS-CoV-2 viral S protein; and (iii) a novel high-throughput surrogate neutralization assay measuring the ability of INO-4800-induced antibodies to block SARS-CoV-2 spike S protein binding to the host ACE2 receptor. Antibodies were detected in the lungs of the vaccinated animals and induced spike-specific T cell responses, thereby demonstrating its potential as a vaccination strategy (Tebas *et al*. 2021). In an open-labeled Phase I clinical trial, INO-4800 demonstrated excellent safety and tolerability, with only Grade 1 adverse effects and no enhanced adverse effects post second dose. Intradermal injection of the vaccine leads to an increased immune response (both humoral and cellular) in 100% (38/38) of the vaccinated subjects. The INO-4800 vaccine candidate is still in phase II/III trials with no results posted, and more than likely it will not receive a EUA or full FDA approval before a time when a significant of population is fully vaccinated.

Symvivo, a clinical stage biotech company based in Canada is developing a proprietary bacTRL-spike vaccine for the prevention of COVID-19 infection. The bacTRL-spike vaccine contains the bacterial medium with either 1, 3, or 10 billion colony-forming-units of live *Bifidobacterium longum* engineered to deliver plasmids encoding spike S protein from SARS-CoV-2. Symvivo has currently initiated a randomized, observer-blind, placebo-controlled phase I clinical trial study (NCT04334980) in healthy individuals with an estimated enrollment of 84 participants, of which 63 randomized healthy individuals will receive the active vaccine in bacterial medium and 21 participants will receive placebo (bacterium media only). This ongoing study will test the safety and tolerability of orally delivered bacTRL-spike vaccine in healthy individuals. The primary objectives of this triple-masked clinical study are to measure the incidence of adverse gastrointestinal-associated reactions to the vaccine as well as to characterize the gastrointestinal immune responses for up to one year post vaccination.

Another, plasmid DNA vaccine candidate nCov developed by Zydus Cadila (company based in India), initiated their phase III trials (CTRI/2020/07/026352). Covigen, a gene-based vaccine developed by Bio-Net Asia (company based in Australia) in collaboration with Technovalia and University of Sydney are planning to initiate their phase I trials (NCT04742842) in Australia after receiving safe and immunogenic profile in their pre-clinical studies. DNA vaccine candidates for COVID-19 have made impressive and promising advancements, but their progress severely lags behind the warp pace of mRNA- and adenovector-based vaccine candidates that are already being administered to millions of people globally. Although DNA vaccines might not contribute to the deterrence of the current COVID-19 pandemic, the testing and clinical trials done now will help to establish the validity of DNA vaccine technology. Currently, DNA vaccines do stand as a promise for any broad use.

Live-attenuated virus

Live-attenuated vaccines contain a weakened form of SARS-CoV-2. This form causes milder infections than those of natural exposures and can induce a sustained robust immune response without causing disease (Jeyanathan *et al*. 2020). The vaccine, administered orally, is expected to infect the intestinal cells, multiply, and provide mucosa-associated immune system sparing the respiratory system (Sanal and Dubey 2020). Also, an ideal live-attenuated vaccine should stimulate enough protective immune responses without reverting to a virulent phenotype. This strategy for long-lasting immunity can aid in the faster development of *"herd immunity"*. Currently, two live-attenuated vaccines are in phase II clinical trials for SARS-CoV-2 infection. The Serum Institute of India, a vaccine manufacturer and global distributor, developed a vaccine COVI-VAC which has just entered phase I clinical trial (NCT04619628) with Codagenix's technology that allows rapid generation of multiple vaccine candidates and uses viral deoptimization to synthesize rationally designed live-attenuated vaccines (Mueller *et al*. 2020). COVI-VAC is an intranasal vaccine candidate designed to develop immunity against all SARS-CoV-2 proteins rather than just spike protein. Also, another promising technology is reverse genetics, which mutates or deletes virulence-related genes and is combined with metagenomics and structural biology to help characterize and predict emergent SARS-CoV-2 viral strains (Ma *et al*. 2020). This technology will facilitate the development of therapeutic strategies to confront future coronavirus outbreaks.

Inactivated virus

Inactivated whole-vaccines are widely used to protect against viral pathogens and for the prevention of emerging respiratory diseases (Sanders, Koldijk and Schuitemaker 2014). Historically, vaccine manufacturers propagate viruses in cells or whole organisms, isolate, concentrate, and purify the viruses prior to chemical or physical inactivation. Compared to live-attenuated viruses, inactivated viruses confer advantages of better safety profiles, are less reactogenic, and easy to manufacture with fewer regulatory hurdles for licensure (Zepp 2010; Sanders, Koldijk and Schuitemaker 2014). The downside of inactivated vaccine is the lower immunogenicity, which suggests the necessity for multiple doses or inclusion of adjuvant such that it may raise formulation complexity as well as production and vaccine costs (Sanders, Koldijk and Schuitemaker 2014).

Preclinical studies in rodents and macaques show varying efficacy for inactivated vaccines. For instance, pilot-scale production of PiCoVacc, a purified inactivated SARS-CoV-2 virus vaccine candidate, elicited SARS-CoV-2-specific neutralizing antibodies in mice, rats, and nonhuman primates against 10 representative SARS-CoV-2 strains. The same study demonstrated protection against SARS-CoV-2 challenge in macaques without any immunopathological exacerbation. In Phase I and II trials started in April 2020 (NCT04352608), participants (18–59 years; phase I, n = 144; phase II, n = 600) were given either a low (3 μg) or high (6 μg) dose per 0.5 mL of aluminum hydroxide diluent per dose of CoronaVac. In both phase I and II trials, incidence of adverse events in the group that received 3 ug dose was lower than that of the 6 ug dose, irrespective of the vaccination schedule cohort (days 0 and 14 cohort or days 0 and 28 cohort). The seroconversion of neutralizing antibodies after the last dose was comparable at both low and high doses in each vaccination schedule cohort, which led the authors to suggest the use of 3 μg dose of CoronaVac for further assessment of vaccine efficacy(Palacios *et al.* 2020; Zhang *et al.* 2021).

Other research trials related to inactivated virus-based vaccine have been conducted or are underway. This includes Sinovac Research and Development Co., Ltd, who conducted their phase III clinical trials (NCT04456595) in July 2020 in Brazil, Turkey, Indonesia, and Chile. Around 25 000 healthy people participated across those four countries (Zhang *et al.* 2021). Out of two stages of phase III trials in Brazil, in first stage participants comprised of health care workers and the general public in the second stage aged between 18 and 59 years. After receiving two-dose vaccination, the efficacy rate was found to be 91.2%. The trials found immunogenicity and was safe, with these promising results, Coronavac has entered phase IV studies (NCT04756830)(Palacios *et al.* 2020).

Likewise, in another study with inactivated viral vaccine, Shangqiu City Liangyuan District Center for Disease developed BBIBP-COV. The vaccine was developed as a 4 weeks interval dual dose IM injection regimen and exhibited safety at test concentrations (2, 4 and 8 μg/dose) in a randomized, double-blind phase I/II trial. (Xia *et al.* 2021). Common adverse reactions were pain and fever. Immunogenicity was witnessed as 100% seroconversion and the development of a robust humoral response. The vaccine was reported to have an efficacy of 79.34%; however, official results are pending. These vaccines were characterized for their immunogenicity by measuring the humoral (RBD and virus specific antibodies) and cellular (IFN-γ secretion in cultures) immune responses at a predetermined time after administration of a specified number(s) of doses. Currently the vaccine is undergoing a phase III trial (NCT04560881) which will provide more insight into safe and efficacy profile. Another, recent phase III study (NCT04641481) virion inactivated vaccine candidate, BBV152 (COVAXIN), developed by Bharat Biotech International Limited, has already been used for emergency use in India (Ella *et al.* 2021) as well as Zimbabwe, Nepal and Mexico.

Protein-based

In August 2020, Novavax biotech company announced positive phase I/II data for NVX- CoV2373 vaccine candidate (NCT04368988). NVX-CoV2373 contains MatrixM™ adjuvant (which provides significant immune response enhancement) and a recombinant SARS-CoV-2 nanoparticle vaccine. The recombinant SARS-CoV-2 is constructed from the full-length wild-type SARS-CoV-2 viral S protein, which mediates viral attachment to the human ACE2 SARS-CoV-2 receptor. After its promising effects to produce high titers of neutralizing antibodies in preclinical studies with rodent and nonhuman primates, a clinical phase I, randomized, observer-blinded, placebo-controlled trial commenced in March 2020. Two doses of NVX-CoV2373 (5 and 25 μg), with or without the MatrixM™ adjuvant was administered intramuscularly, 21 days apart. The vaccine was generally well-tolerated and had reassuring safety profiles when tested on 131 healthy individuals (ages 18–59), as it elicited a greater neutralizing antibody response after a second vaccine dose. The magnitude of antibody responses was also comparable to those detected in convalescent sera of hospitalized patients. Taken together, NVX-CoV2373 is a potential candidate and further efficacy studies are warranted. A phase III trial commenced based on phase I/II preliminary results (NCT04611802). Novavax, Inc. announced that NVX-CoV2373 met the primary endpoint, with a vaccine efficacy of 89.3%, in its Phase III clinical trial conducted in the United Kingdom (UK) against the-predominant UK variant at that time (Novavax 2021). Novavax has also announced successful results of its Phase II/b study conducted in South Africa. In HIV-1 seronegative population (94% of the study population), 60% efficacy for the prevention of mild, moderate, and severe COVID-19 disease was observed. Post hoc vaccine efficacy against B.1.351 was 51% among the HIV-1 seronegative participants (Shinde *et al.* 2021). Whether NVX-CoV2373 could completely protect against the globally spreading South Africa variant is questionable, yet investigators stress its value in reducing COVID-19 severity (Kunzmann 2021). Phase III, randomized, placebo-controlled, observer-blinded study in the US and Mexico is also ongoing to evaluate the efficacy, safety, and immunogenicity of NVX-CoV2373 with Matrix-M in up to 30, 000 subjects 18 years of age and older compared with placebo. Taken together, protein-based vaccines are both being developed and implemented across multiple nations.

Moreover, as part of accelerated SARS-CoV-2 vaccines development, SCB-2019 vaccine elicited robust humoral and cellular immune responses against SARS-CoV-2, with high viral neutralizing activity (Richmond *et al.* 2021). SCB-2019 vaccine comprises S-Trimer protein which uses the Trimer-Tag protein (Liu *et al.* 2017), the protein derived from the C-terminus of human type I procollagen, which preserves the trimeric conformation of the SARS-CoV-2 spike protein. This vaccine differs from those already approved because of the use of Trimer-tag protein that has not previously been used in clinical trials. SCB-2019 is formulated with two different adjuvants (either AS03 or CpG/Alum), that are well tolerated, and thus are considered suitable for further clinical development.

Overall, to ensure the safety and efficacy of each long-term confidence in these and other vaccination programs, continuous monitoring for adverse side effects, efficacy against viral mutations, and longevity of memory responses, are all required in all immunized populations. Owing to enhanced availability, data from randomized, placebo-controlled clinical vaccine trials will be required for testing against new viral variants. Measurement of the effects of new and current vaccines against such SARS-CoV-2 variants require extensive genomic surveillance, detailed "correlate of protection" evaluations, and robust surveillance and viral sequencing (Neuzil 2021).

Pregnancy

As of March 1 2021, more than 73,600 COVID-19 infections and 80 maternal deaths of pregnant women have occurred

in the United States. SARS-CoV-2 infection is severe in pregnant women compared to their nonpregnant counterparts. Despite the higher risk, pregnant and lactating women were not included in the COVID-19 vaccine trials reportedly due to heightened safety concerns. Thus data is currently lacking in regards to vaccine efficacy and the degree of humoral protection available in this population that includes neonates (Gray *et al.* 2021).

To address the vital question of whether pregnant and lactating women elicit an immunogenic response to the COVID-19 vaccine, a group led by scientists and doctors of Boston conducted a prospective cohort study with reproductive-age vaccine recipients (84 pregnant, 31 lactating, and 16 non pregnant) at two academic medical centers.

The study observed that pregnant and lactating women elicited comparable vaccine-induced humoral immune responses to nonpregnant controls. They also generated higher antibody titers than those observed following SARS-CoV-2 infection during pregnancy. Vaccine-generated antibodies were also present in umbilical cord blood and breast milk after maternal vaccination. Titers of IgG, IgA, and IgM to SARS-CoV-2 viral S protein and RBD were quantified in the sera of participants (n = 131) and breast milk (n = 31) at baseline, second vaccine dose, 2–6 weeks post the second vaccine, and at delivery. The antibody titres induced through vaccination were equivalent in pregnant and lactating women in comparison to non-pregnant women (median [interquartile range] -5.62 [4.77-5.98] non-pregnant, 5.59 [4.68-5.89] pregnant, 5.74 [5.06-6.22] lactating, $p = 0.24$). Titers were significantly higher than those caused by SARS-CoV-2 infection during pregnancy ($p < 0.0001$). Vaccine-generated antibodies were present in all umbilical cord blood and breast milk samples. Neutralizing antibody titers were lower in the umbilical cord compared to maternal sera. According to the study, the COVID-19 mRNA vaccines generated robust humoral immunity in pregnant and lactating women, with immunogenicity and reactogenicity similar to what has been observed in nonpregnant women. It was also observed that vaccine-induced immune responses were more significant than the response to natural infection. Also, immune transfer to neonates occurred via the placenta and breast milk (Gray *et al.* 2021).

Children

As of March 18 2021 the American Academy of Pediatrics estimate that 3.34 million children in the U.S. had been infected with COVID-19, thousands were hospitalized, and over 260 children died. Younger kids are less likely to get COVID-19 and spread it than adults, but adolescents look more like adults in terms of acquiring COVID-19 and spreading it. It is important to note that vaccination of children is pivotal to reach herd immunity (Pediatrics 2021). As of March 29 2021, the two companies that received *Emergency Use Authorization (EUA)*, Pfizer-BioNTech and Moderna, have launched clinical trials of the same COVID-19 vaccines (different doses) in children as young as 6 months, older children, and teenagers. As of now, there is insufficient data regarding the side effects of vaccination in children. On March 15 2021, Moderna started a new phase II/III, 2-part clinical trial, KidCOVE to study the effectiveness of mRNA-1273 in children aged 6 months to less than 12 years (NCT04796896). The trial is to test two or three age-dependent dose levels, administered in two shots 28 days apart. Moderna plans to enroll about 6,750 children in the U.S. and Canada for this trial, with some children getting the vaccine and others a placebo. In December 2020, Moderna started a Phase II/III study with 3,000 participants 12 to less than 18 years of age to evaluate the safety and reactogenicity of a single dose level of mRNA-1273 vaccine administered in 2 doses 28 days apart to an adolescent population in comparison to placebo (NCT04649151).

In a phase III trial of 2,260 participants aged between 12 and 15 years, (NCT04713553) the BNT162b2 vaccine demonstrated 100% efficacy and robust antibody responses, exceeding those reported in trial of vaccinated 16–25 year old participants in an earlier analysis. In the trial, only 18 cases of COVID-19 were observed in the placebo group versus none in the vaccinated group. Vaccination with BNT162b2 elicited SARS-CoV-2–nAbs geometric mean titers (GMTs) of 1239.5, demonstrating strong immunogenicity in a subset of adolescents one month after the second dose. Further, BNT162b2 administration was well tolerated, with side effects generally consistent with those observed in participants 16 to 25 years of age (Pitts 2021).

On March 25 2021, Pfizer announced plans to launch a phase I/II/III study of 4,644 children ages 6 months to 12 years (NCT04816643). They are to be administered upto 3 different dose levels in each age group to study safety and efficacy.

SUMMARY

The COVID-19 global pandemic is defined by unique immunologic profiles. These profiles define antiviral host immunity and spread of disease. The means to contain the pandemic reside in effective vaccination providing robust immune protection for the individual and society. Multiple vaccine strategies are offered that include attenuated virus and DNA, RNA and protein subunits. The Moderna and Pfizer/BioNTech's vaccines have reached multiple nations and are broadly administered. Long-term immunity will require an even better understanding of virus-host interactions. Therapeutic targets are being defined as immunopathogenic viral mechanisms get defined. The human immune response to SARS-CoV-2, while complex, supports the interplay between innate and adaptive immunity that affect a broad range of pro-inflammatory and signaling pathways. This in turn leads to end-organ damage and accelerated disease. The complexity is defined by the emergence of viral variants, time, and populations. Research thus far has dissected infection at the molecular and cellular level. Immune therapies for SARS-CoV-2 infection will continue to evolve and focus on more effective generation of humoral and T cell activities that can provide more durable antiviral activities. Since the firsr COVID-19 diagnosis, significant research advances were made to contain the pandemic. Nevertheless, future works must be done before the global COVID-19 threat is permanently eliminated.

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