Short Note

A SARS-CoV-2 overview for people in a hurry

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Abstract   SARS-CoV-2 is the etiologic agent of the current COVID-19 pandemic which has wreaked unprecedented economic and healthcare calamity. It is a deadly virus belonging to the Coronaviridae family, with high sequence similarity to the 2003 SARS epidemic coronavirus[1]. The global race to produce vaccines to stem the disease — as well as the public health urgency — has spurred tremendous growth in the litany of literature which attempts to uncover the enigma of this deadly virus. Amidst this evergrowing list of literature, this paper seeks to concisely elaborate on key progresses made in the understanding of SARS-CoV-2 in the realms of its life cycle, epidemiology, methods for detection, and vaccine research into an easily assimilable paper for readers.

Viral Life Cycle   SARS-CoV-2 is a Betacoronavirus that expresses spike (S) glycoproteins on its viral envelope which are crucial for viral entry[2]. The spike protein is a trimeric protein, with each monomer containing an ectodomain, a transmembrane anchor, and an intracellular tail[3]. Most importantly, the ectodomain comprises two subunits: the S1 subunit for viral attachment, and the S2 subunit for membrane fusion[4]. SARS-CoV-2 attachment begins with the S1 subunit C-terminal domain (CTD) recognising and binding angiotensin converting enzyme 2 (ACE2) receptors and sugar receptors on cell surfaces[5]. The CTD of the S1 subunit has two conformations with differing affinity to ACE2, and this conformation depends on whether furin cleaves the S protein at the S1/S2 proprotein convertase (PPC) motif during viral biosynthesis. The higher affinity 'up' conformation is active when Golgi-resident furin cleaves the PPC motif, while the lower affinity 'down' conformation is kept when the PPC motif

is not cleaved[6].

SARS-CoV-2 viral entry can be achieved through direct membrane fusion or endocytosis[7]. Membrane fusion is a fundamental process required in both entry methods, requiring the S2' site on the S2 subunit to be proteolytically cleaved by host cell serine proteases. This proteolytic cleavage causes the S2 subunit to fold its fusion peptide into a hairpin that binds the target membrane. Thereafter, the heptad repeats HR1 and HR2 in the S2 subunit form a 6-helical bundle that merges the viral envelope with either the host cell or lysosomal membrane. In the case of membrane fusion, host cell-surface Transmembrane Protease, Serine 2 (TMPRSS2) will proteolytically activate the S2 subunit at the host cell surface for direct membrane fusion[8]. For cells lacking TMPRSS2, endocytosis occurs after ACE2 binding. In the proposed endocytic pathway, the maturation of the endosome involves acidification of the endosomal environment, which in turn activates the serine protease cathepsin L. This then leads to the merger of the viral envelope with the lysosomal membrane, allowing viral nucleocapsid release into the cytosol[3,9].

The viral genome (gRNA) is a positive-sense ssRNA with features resembling a typical mRNA, such as a 5' cap and 3'-poly(A) tail, allowing it to be directly translated. The 5' end of the gRNA contains 16 non-structural proteins (Nsps 1-16) encoded by the reading frames polyprotein (pp) 1a and 1ab, with pp1ab encoding all 16 Nsps necessary to form the replicase complex. The 3' end encodes the structural proteins spike, envelope, matrix and nucleocapsid (S, E, M, and N) necessary for producing new virions[10]. Upon cytoplasm entry, pp1a and pp1ab are translated by hijacking the host translation machinery to yield two polyproteins. Next, pp1a and pp1ab must be proteolytically cleaved to obtain isolated nsp fragments, and this occurs in two steps[10]: the proteases Nsp3 (PLpro) and Nsp5 (3CLpro) first auto-process themselves before they go on to proteolytically separate other nsp proteins through recognition cleavage sequences located at their boundaries[11]. Nsp12, the RNA-dependent RNA polymerase (RdRp) then combines with its cofactors nsp7 and nsp8 to form a replication-transcription complex (RTC) to kickstart RNA synthesis. Viral RNA synthesis generates highly immunogenic dsRNAs, because RdRp utilizes the viral genome as a template. To evade host Toll-like receptor detection, SARS-CoV-2 synthesises its new RNA inside double membraned vesicles (DMVs) formed through modifying the host's endoplasmic reticulum[12]. Thereafter, viral gRNA is used for continuous synthesis of full-length negative-sense RNA and discontinuous synthesis of short subgenomic RNAs (sgRNAs). These are then used for transcribing

the viral genome, as well as structural and accessory protein mRNAs respectively[13]. The synthesised mRNAs are then released into the cytosol through nsp3 channels formed on the DMV membrane[14]. In the cytosol, the viral structural proteins S, E, and M are translated and inserted into the membrane of the endoplasmic reticulum (ER). Simultaneously, the translated N protein binds to viral gRNA through its N-terminal domain, and then use its oligomerising C-terminal domain to assemble the gRNA into a long helical nucleocapsid structure crucial for viability and getting translated in the next infectious cycle[15]. The assembly of the N protein first involves N protein dimerisation, followed by dimer assembly into the higher order capsid structure[16]. The S, E, and M proteins then migrate into the ER-Golgi intermediate complex (ERGIC) to bud off within exocytic vesicles, with the long helical viral nucleocapsid entering to complete viral assembly and maturation for exocytosis[17].

Epidemiology In early January 2020, the first SARS-CoV-2 genome was published. This genomic strain (L strain), which was taken as the reference genome, was obtained from an individual who was infected at the Huanan Seafood Market in Wuhan City. Subsequently, many other genomes have been sequenced and published globally, and a mass genomic screen of 48,635 global SARS-CoV-2 genomic samples revealed single nucleotide polymorphisms (SNPs) to be the most prevalent form of mutation. The screen revealed several SNPs to be highly conserved, which allowed the delineating of specific strains that arose from mutations to the ancestral L strain[18]. The first variants of the L strain started surfacing shortly after the first published sequence; the S strain appeared in early January, with its distinctive SNPs C8782T and T28144C. Shortly after, the G and V strains appeared in mid-January 2020, with the G strain characterized by its 4 SNPs; C2411T, C3037T, C14408T and A23403G, while the V strain has the characteristic G11083T and G26144T SNPs. In late-February 2020, the G strain attained two more mutations, G25563T and GGG28881AAC, giving rise to the GH and GR strains respectively[18]. Recently, minks in Denmark were also noted to have been infected by SARS-CoV-2[19,20]. Perhaps more pressingly, in December 2020, the UK has reported a new variant of SARS-CoV-2 termed variant B.1.1.7 (or Variant Under Investigation, VUI202012/01)[21]. Variant B.1.1.7 is distinguished by 23 mutations, including the N501Y mutation in the receptor binding domain of the SARS-CoV-2 spike protein[21]. Variant B.1.1.7 also possesses a six-nucleotide deletion in the spike protein gene, resulting in the loss of amino acids 69 and 70 (Δ69/70). Preliminary investigations suggest that Δ69/70 enhances viral infectiv-

ity[22] and may lead to a false negative signal for the spike protein gene during PCR testing.[23,24].

Globally, the most prevalent strain is the GR strain. However, continentally, the dominant strain differs. For instance, North American cases are largely from the GH strain, although the S strain still accounts for a significant number of cases, whereas Europe and South America are predominantly infected with the GR strain. Africa is predominantly infected with the G strain, while Asian cases are mostly from the L strain. Interestingly, Oceania cases appear to have a balanced distribution of all known SARS-CoV-2 strains[18]. Most importantly, the G, GH and GR strains are becoming the dominant strain in all continents with a simultaneous decrease in L and V strain infections. It is possible that the G, GH and GR strains' increased prevalence is due to an evolutionary advantage conferred by its distinctive A23403G SNP which is known to confers some structural changes to the S protein[25]. Interestingly, despite the global ubiquity of the G, GH and GR strains, China reported almost no instances of these strains in its sequenced genome database[18]. Finally, apart from the characteristic SNPs which define specific strains, there are many other arbitrary SNPs that appear but are not prevalent enough to be classified into a strain, and these are classified as "others", or O strains[18].

Detection   SARS-CoV-2 can be detected in a patient in two main ways — by assaying for viral RNA, or by detecting SARS-CoV-2 specific antibodies using serological methods. In the case of RNA-based detection, multiplex RT-PCR is the current gold standard[26,27]. In RT-PCR, reverse transcription first converts viral RNA to DNA, before primers specific to several SARS-CoV-2 genes hybridise to the reverse-transcribed DNA to undergo PCR. During PCR, probes containing a quenched fluorophore hybridise to a gene segment located between a forward and reverse primer. These probes get cleaved by DNA polymerase, releasing the quencher from the fluorophore and creating a fluorescent signal which is read by the PCR cycler to indicate the presence of viral RNA[28]. Most commercial SARS-CoV-2 PCR kits test for SARS-CoV-2 structural protein genes (S, E, M, N) and a positive control, human RNAse P. This positive control is especially important for detecting false negatives due to problems with the PCR reaction[26]. Although RT-PCR is highly specific and can detect the virus in presymptomatic patients, it cannot detect low viral loads in recovered patients and hence is commonly used only as a diagnostic tool. The labour-intensiveness of RNA extraction also makes high throughput population testing difficult[26,28]. For this reason, serology-based assays addresses these limitations albeit with reduced sensitivity and specificity.

Serological assays detect antibodies such as IgG and IgM or neutralizing antibodies that are synthesised in response to viral exposure. IgG and IgM both recognize SARS-CoV-2's immunogenic portions such as the S and N protein[26]. This can be achieved through lateral flow assays (LFA), enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunosorbent assay (CLIA). LFAs utilise patient blood samples which can be obtained quickly through a finger prick and applying it to a chamber containing a nitrocellulose membrane which registers a colorimetric change indicative of IgG/IgM presence[26]. ELISA is run by first applying patient whole blood/serum to wells that contain antigens to capture IgG and/or IgM before a secondary antibody is added which binds to IgG/IgM. The secondary antibody catalyzes a chromogenic reaction which indicates antibody presence[26,29]. CLIA is similar to ELISA, except with shorter incubation times and higher sensitivity[26,29]. Neutralizing antibodies can also be detected using plaque reduction neutralization tests[30]. In general, although serological tests have lower sensitivity and specificity than PCR-based assays, they are faster to conduct and are better suited for high throughput testing in large populations.

Different stages of the infection require different detection methods; IgM and IgG production does not occur immediately in the early stages of infection (taking ~5 and ~14 days respectively[31]), thus RT-PCR is necessary as a diagnostic tool. However, in the recovery/recovered phase, viral RNA count is low and might not be detected by RT-PCR. Serological tests are thus necessary, and the combination of both RT-PCR and serological tests provide the best insight into the state of infection control in a community.

Pathogenesis Amongst SARS-CoV-2 infected cases, approximately 80% have mild or no symptoms. Of the 20% of patients who require hospitalization, a quarter require intensive care often requiring ventilators[32]. In such severe clinical cases, the virus enters the respiratory tract and targets alveolar endothelial cells (AECs) such as type I and type II pneumocytes to establish a viral loop that drives virion replication. It does this by inhibiting host interferon (INF) synthesis and signalling pathways which are critical to halting SARS-CoV-2 replication in the early stages of infection. Firstly, SARS-CoV-2 avoids triggering host Toll-like Receptors (TLRs) that would lead to interferon secretion by hiding itself in the DMVs, hiding its dsRNA intermediates that are ligands for host TLRs. Secondly, SARS-CoV-2 also antagonizes the INF response, stymying INF-induced signal transduction pathways involved in the upregu-

lation of antiviral proteins within AECs[32,33]. In fact, SARS-CoV-2 has an increased ability to suppress IFN induction as compared to SARS-CoV, attributed to a premature stop codon in ORF3b[34], thus allowing for viral replication without a significant induction of all IFN types (I, II, or III)[32,35]. These mechanisms act in synergy to allow viral replication to proceed unhindered, causing an outburst of new virions shortly after initial infection. A vicious cycle ensues — new SARS-CoV-2 particles infect more AECs to replicate whilst suppressing the host antiviral IFN response. This eventually leads to cytopathy in the form of syncytium formation and widespread pyroptosis of AECs in a bid to halt viral replication[30].

Pyroptosis of infected AECs results in the release of massive amounts of proinflammatory cytokines such as interleukin-6 (IL-6), granulocyte-colony stimulating factor (G-CSF), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein (MIP)1α, and tumour necrosis factor (TNF)-α[36]. TNF-α and IL-6 both negatively regulate T-cell survival and or proliferation[37], resulting in decreased numbers of T cells in SARS-CoV-2 infected patients. This results in lymphopenia especially with regards to peripheral blood cells[36,38]. Programmed cell death protein (PD)-1 and T-cell immunoglobulin mucin (Tim)-3 expression are also increased due to

SARS-CoV-2 – these markers of T cell exhaustion implies the impairment of proper T cell function[36]. Alternatively, T cell counts may also be negatively affected by the direct induction of T cell apoptosis by SARS-CoV-2[39,40]. Everything considered, as T cells serve to dampen hyperreactive innate immune responses, the depletion of T cells results in damage to host tissues. In addition, the remaining CD8+ T cells were observed to have an increased concentration of cytotoxic granules, which accounts for the immune injury caused by SARS-CoV-2 infection[36]. This combination of adaptive and innate host immune disruption could explain the many opportunistic infections that occur alongside SARS-CoV-2 infection.

Additionally, SARS-CoV-2 also damages the lung by disrupting the inflammation-regulating renin-angiotensin (RAS) pathway. In the RAS pathway, ACE converts AngI into AngII, a pro-inflammatory hormone. ACE2 on the other hand, converts AngI into Ang-(1-9) as well as AngII to Ang-(1-7). Ang-(1-7) binds to MasR to counter-regulate AngII-mediated inflammation[32]. However, viral entry into AECs causes depletion of ACE2 on the cell surface, leading to AngII build-up in lung cells. Elucidating further, the build-up of AngII is caused by: (i) the decrease in AngI conversion to Ang-(1-9) and (ii) the decrease in AngII conversion in Ang-(1-7). This results in an array

of AngII-mediated inflammation and other tissue injury pathways such as free radical synthesis, exacerbating the already-damaged lung from the aforementioned pneumonia[41].

Moreover, the apoptosis of epithelial cells and the cytokine storm induced by SARS-CoV-2 exhibits prothrombotic effects[32]. The ACE2/Ang-(1-7)/Mas1R pathway exhibits an anti-thrombotic effect, but is disabled by SARS-CoV-2 infection as detailed above. Together, this results in pulmonary thrombosis, which in turn impairs gaseous exchange or causes acute lung injury.

Vaccines Vaccines prime an individual's immune system by exposing immunogenic portions of the virus such as SARS-CoV-2's S protein to the patient. This primes the immune system to produce neutralising antibodies, memory T-cells, or even enhance innate immunity to swiftly eradicate SARS-CoV-2 upon future exposure[42]. Other immunogenic portions of SARS-CoV-2 that can be exploited for targeting by vaccines include the other structural proteins M, E and N. Several methods for exposing the immunogenic portions of SARS-CoV-2 could be achieved, each with its own benefits and limitations.

Nucleic acid-based vaccines utilise DNA or RNA that encode whole or fragmented S proteins. Inoculating the DNA into a patient can be done using various methods — one innovative method is Inovio's INO-4800, which utilizes a patented electroporation technology to deliver S protein-encoding plasmids into the muscle tissue of patients[43]. The S protein then gets expressed for immune priming. RNA vaccines utilise mRNA encoding S proteins, such as Moderna's mRNA-1273 vaccine. This method utilises the encapsulation of mRNA encoding SARS-CoV-2 S proteins inside lipid nanovesicles that are injected into patients. The mRNA is eventually expressed in the lymph nodes where it primes the immune system to secrete neutralising antibodies[44]. No other nucleic-acid based vaccines had been approved for clinical usage until the U.S Federal Drug and Food administration granted emergency use approval for both Pfizer-BioNTech and Moderna's mRNA vaccines, collectively representing the first nucleic acid vaccines to be approved for clinical usage[45,46]. Most importantly, it is believed that nucleic-acid based vaccines can be synthesised more quickly than other types of vaccines, a critical consideration for meeting high global demand[47]. However, one caveat to nucleic-acid vaccines like DNA vaccines is the possibility for the foreign DNA to be recombined into host somatic cell genome, leading to undesired mutations[48].

Protein vaccines directly utilise recombinantly expressed S proteins as the immunogen to prime the im-

mune system. An example is Novavax NVX-CoV373 which synthesizes full length SARS-CoV-2 S proteins into nanoparticles. These nanoparticles are then injected intramuscularly alongside a potent adjuvant which drives the migration of leukocytes like T and B cells, as well as NK and dendritic cells into the lymph nodes where they get exposed to the S protein and start synthesising neutralizing antibodies[48,49]. Another method utilises pre-fusion S proteins which are highly immunogenic but are structurally unstable, and hence require stabilization through novel technologies such as molecular clamping before introduction into a live host for immune priming[50]. However, protein vaccines require a thorough understanding of the specific viral molecules that induce the immune response, without which they cannot be synthesised. Fortunately, protein-based vaccines are generally cheaper to manufacture than traditional attenuated vaccines[51].

Whole inactivated vaccines are traditional vaccines believed to elicit the strongest immune response as it involves administering whole viruses that contain all the possible immunogens and yet are replication-deficient. One example is PicoV-acc from Sinovac, which involves inactivating SARS-CoV-2 with β-propiolactone, which modifies viral RNA to make it replication-deficient[52]. Picovac is then administered intramuscularly with an adju-

vant to prime an immune response. However, whole inactivated vaccines require lengthy and extensive testing to ensure that the inactivated virus is unable to revert to a pathogenic state. It is however, believed to also be the most efficacious.

Finally, viral vectors are also used, in which non SARS-CoV-2 viruses can be engineered to express the S protein but do not cause disease. For instance, ChAdOx1 and Ad5-nCoV by AstraZeneca and CanSino Biologics respectively both utilise simian adenoviruses that cannot replicate or cause disease but yet encode and produce full-length S proteins on the viral surface to prime the immune system[53,54]. One advantage of adenoviral vaccines is the ease with which adenoviruses can be engineered to express immunogenic proteins, making them easily adaptable to mimic different immunogenic portions of SARS-CoV-2. However, pre-existing immunity against the viral vector can lead to an immune response against the viral vector without any regard for the extra SARS-CoV-2 protein present on the adenovirus, hence leading to a failure to prime immunity[55].

Finally, a caveat to vaccine development: coronaviruses are able to make use of antibody dependent enhancements (ADE), utilising anti-S protein IgG to facilitate virus-IgG uptake via Fc receptors[56]. This potentially causes viral infection in cells of the

immune system (such as macrophages or neutrophils). More research is required to confirm ADE in SARS-CoV-2 infection and care should be taken to ensure that the antibodies produced post-vaccination do not contribute to ADE, either by removing or inactivating regions associated with ADE[57]. Of course, long-term monitoring of vaccinated individuals must also be established, to properly assess the risk of ADE.

Conclusion The COVID-19 pandemic represents one of mankind's biggest challenges. This essay has outlined the crucial facts of the infectious cycle of SARS-CoV-2, as well as methods to detect and deter the virus which represent paradigms to stem the pandemic. Besides diagnosing and treating those who are infected, contact tracing, increased vigilance in hygiene practices, as well as quarantining infected patients are other methods to halt the spread of this virus.

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