COVID-19

A biological summary

Abstract

This report summarises information from scientific papers into reviews of what we currently understand about the biology of the virus SARS-CoV-2, covering the biochemistry of the structure and pathogenesis of the virus, cellular interactions and immune system, virus prevention, and possible treatments for this virus.

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Introduction

COVID-19, aka Coronavirus disease 2019 is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)[1]. Common symptoms of the virus may include fever, tiredness, dry cough, and shortness of breath[2], and most of the cases result in mild symptoms of viral pneumonia and organ failure[3]. Chronic patients suffering from COVID-19 might experience acute myocardial injury and chronic damage to the cardiovascular system[4].

The virus outbreak was first identified in Wuhan, China, in December 2019[5], and it's a zoonosis that had Spillover infection from an animal origin to humans. The virus is commonly spread between people in close contact via droplets from coughing, sneezing or spitting while talking[6]. Also, people can be infected by touching their faces after touching surfaces that are contaminated by droplets falling on it, as the virus can survive on surfaces for more than 72 hours[7].

[1] Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. (March 2020). "The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2". Nature Microbiology.

[2] Coronavirus symptoms, World Health Organization (WHO, Archived from the original on 16/04/20 https://www.who.int/health-topics/coronavirus#tab=tab_3

[3] "Q&A on coronaviruses". World Health Organization (WHO). Archived from the original on 20 January 2020. Retrieved 27 January 2020.

[4] Zheng YY, Ma YT, Zhang JY, Xie X (May 2020). "COVID-19 and the cardiovascular system".

[5] "WHO | Novel Coronavirus – China". WHO. Retrieved 9 April 2020.

[6] Berger K (12 March 2020). "The Man Who Saw the Pandemic Coming". Nautilus. Archived from the original on 15 March 2020. Retrieved 16 March 2020

[7] "New coronavirus stable for hours on surfaces". National Institutes of Health. 17 March 2020. Archived from the original on 23 March 2020. Retrieved 23 March 2020.

Structure Of SARS-CoV-2

SARS-CoV-2 is a spherical virus which has an average diameter of around 120 nm[8]. The virus has 4 structural proteins:

- Spike(S)
- Envelope(E)
- Membrane(M)
- Nucleocapsid(N)

Nucleocapsid(N) is the protein that surrounds the virus RNA, which has genetic information that goes into the human body cell and multiplies from transcribing on ribosomes on the human cell. The Envelope protein(E) are protein channels near the membrane(M) protein and spike(S) protein[9]. The membrane protein is made of phospholipid bilayer and surface proteins. The Spike protein on the viral envelope pops out and it is responsible for binding into complementary receptors on human body cells.

Understanding the structure of SARS-CoV-2 is very important for developing a treatment, as most of the antivirals rely on targeting specific protein receptors or interacting with certain mechanisms in the virus.

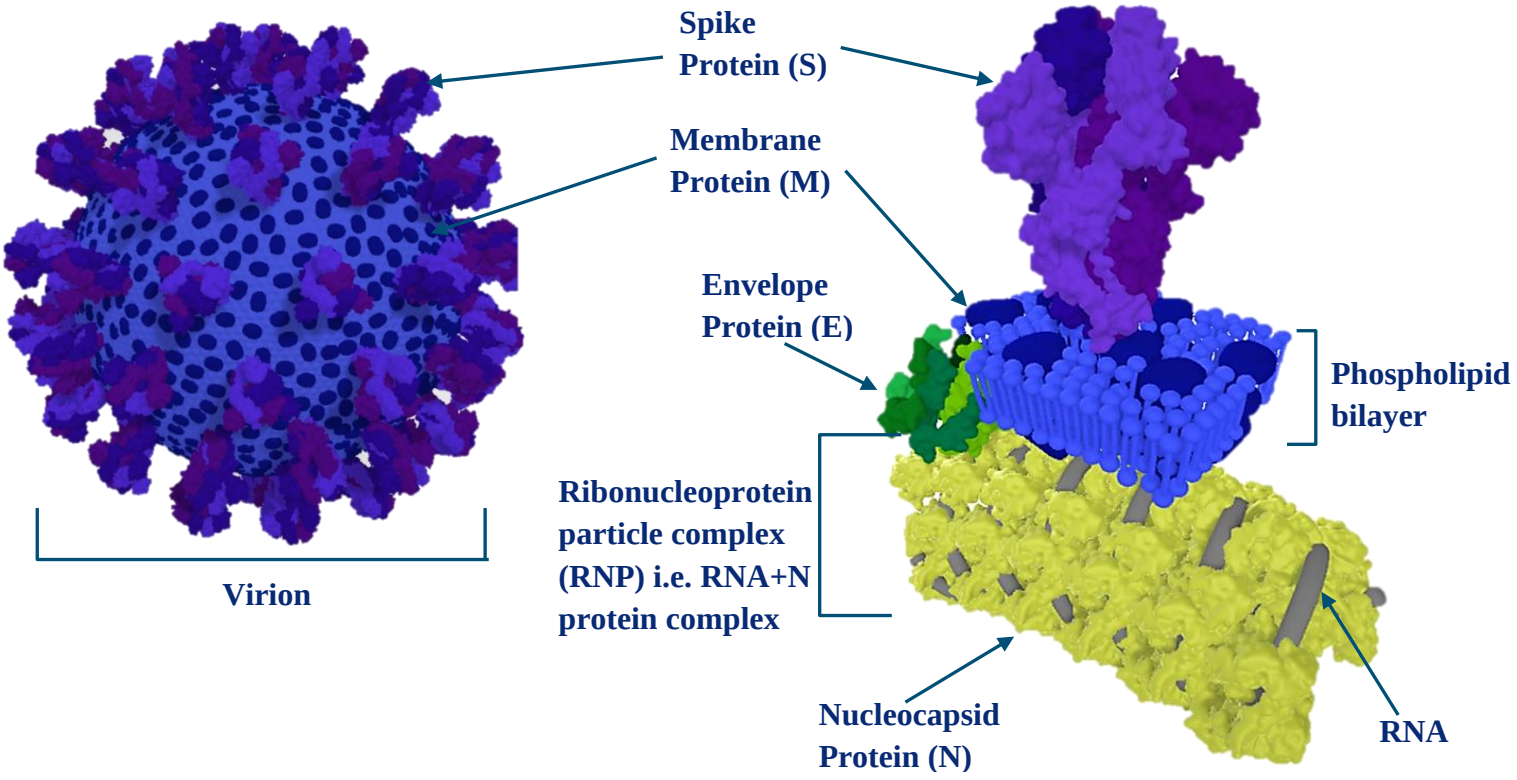


Figure 1 Diagram of the structure of SARS-CoV-2

[8] Fehr AR, Perlman S (2015). Maier HJ, Bickerton E, Britton P (eds.). "Coronaviruses: an overview of their replication and pathogenesis"

[9] Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. (February 2020). "Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods"

Figure S1- SARS CoV-2 Envelope Section and SARS CoV-2 Virion, Glasgow life science, April 2020, https://sketchfab.com/3d-models/sars-cov-2-envelope-section-400b397b402246eaab2d33436f43bcb5

Spike Protein

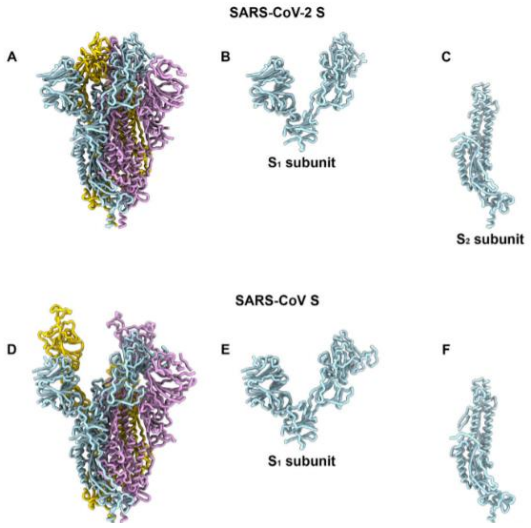


Figure 2 Comparison of the 3D protein structure of spike protein of SARS-CoV-2 and SARS-CoV

The spike protein (S) shown above sticks out the lipid bilayer resembling a crown hence the name corona. The S protein is a trimer (3 protein units) composed of a short intracellular tail, a transmembrane anchor, and a large ectodomain (part of the protein that extends into extracellular space) that consists of a receptor binding S1 subunit and a membrane-fusing S2 subunit[10]. The spike protein has two forms, pre-fusion and post-fusion. The post-fusion form is a metastable state i.e. transitioning from post-fusion back to the pre-fusion structure is thermodynamically & kinetically unfavourable and hence irreversible (large Ea.)[11].

Angiotensin-converting enzyme 2 (ACE2) is said to be the binding site of SARS-CoV-2. ACE2 is a membrane-associated aminopeptidase expressed in vascular endothelia, renal and cardiovascular tissue cells, and epithelia of the small intestine and testes[12] . ACE2 is highly expressed on the luminal surface of intestinal epithelial cells, functioning as a co-receptor for nutrient uptake, and amino acid resorption from food[13]. It is also highly expressed in Alveolar type II epithelial cells which is why both the intestines and the lungs are the focus of concern for viral invasion. The portion of the extracellular region of ACE2 that includes the first α-helix, lysine 353 residues (354th lysine R groups) and proximal residues (R groups closest to the receptor-binding domain) of the NH2 terminus of β-sheet 5 (5th beta-sheet), interact with high affinity with a specific peptide sequence called the receptor-binding domain (RBD) of the SARS-CoV S protein[14]. The S protein of SARS-CoV-2 has slightly different residues(R groups) to SARS-CoV that allow more protein-protein interactions/contacts(PPI/PPC), so the binding process requires a lower activation enthalpy/energy (more energetically favourable and more -ve ΔG), and a smaller dissociation constant Kd (how easily a protein complex breaks down) of 5×10-12 compared to SARS-CoV (1.1×10-10) and other coronaviruses at room temperature 298K/25.0°C (temperature at which the Kd are valid)[15], and hence a stronger affinity between the S protein and ACE2 compared to other coronaviruses. The S protein has also been found to be less thermodynamically stable than other CoVs (more reactive), requiring a lower temperature and shorter time period for activation[16]. These factors could be part of the reason for the rapid transmission and stronger virulence of SARS-CoV-2.

[10] Colman, P., Lawrence, M. The structural biology of type I viral membrane fusion. Nat Rev Mol Cell Biol 4, 309–319 (2003). https://doi.org/10.1038/nrm1076

[11] Walls AC, Tortorici MA, Snijder J, Xiong X, Bosch BJ, Rey FA, et al. Tectonic conformational changes of a coronavirus spike glycoprotein promote membrane fusion. Proc Natl Acad Sci U S A. 2017;114(42):11157–62. Epub 2017/10/27.

[12] Hamming, I., W. Timens, M. L. Bulthuis, A. T. Lely, G. J. Navis, and H. van Goor. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J. Pathol.

[13] Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM (2012) ACE2 links amino acid malnutrition to microbial ecology andf intestinal inflammation.

[14] Li, W., C. Zhang, J. Sui, J. H. Kuhn, M. J. Moore, S. Luo, S. K. Wong, I. C. Huang, K. Xu, N. Vasilieva, A. Murakami, Y. He, W. A. Marasco, Y. Guan, H. Choe, and M. Farzan. 2005. Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2.

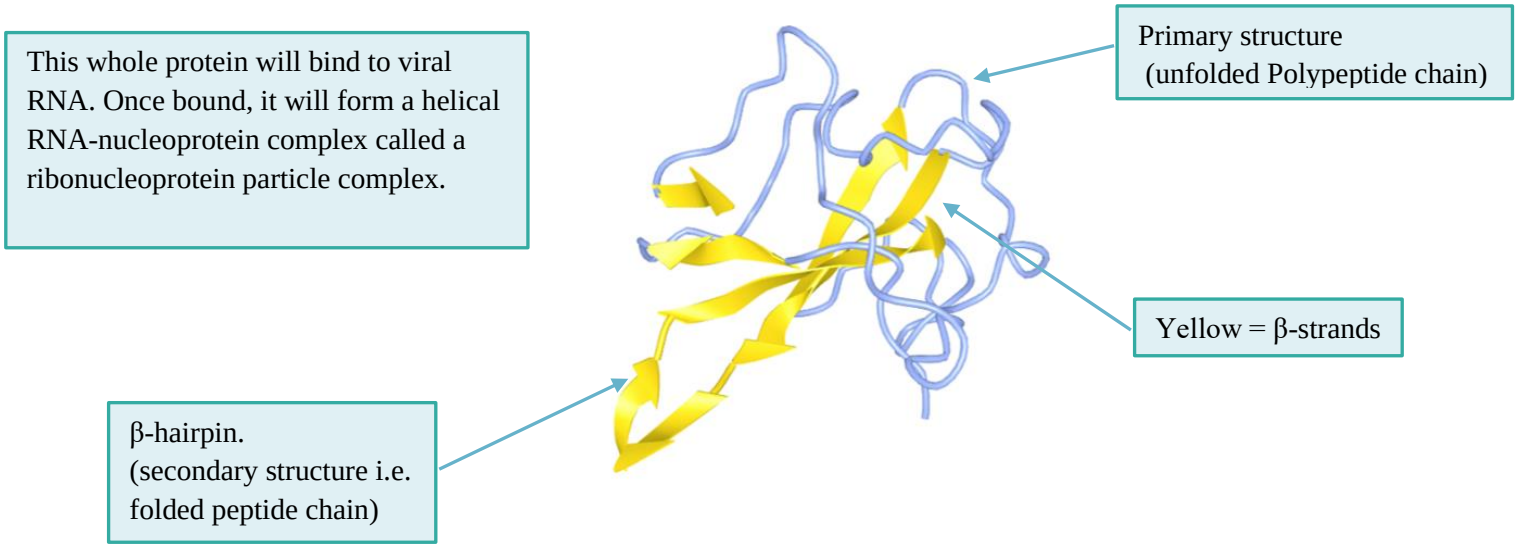
[15] Joseph Thomas Ortega, H., 2020. Role Of Changes In SARS-Cov-2 Spike Protein In The Interaction With The Human ACE2 Receptor: An In Silico Analysis.

[16] Ou, Xiuyuan et al. "Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV." Nature communications vol. 11,1 1620. 27 Mar. 2020

Nucleocapsid protein

Nucleocapsid = viral nucleic acid bonded to a capsid protein (nucleocapsid protein i.e. N protein). The N protein is a multifunctional protein with complex roles in viral assembly, virus budding/envelope formation, and cell signalling[17]

Figure S3: 3D structure of Nucleocapsid protein



The SARS-CoV-2 nucleocapsid (N) protein is an RNA binding protein. The N protein interacts via an N-NTD binding site, (a sequence of amino acid residues at the free NH2 end of the N protein polypeptide) with ribonucleotides in the viral RNA[18]. Once the viral RNA binds to the N-NTD of the N protein, a helical ribonucleoprotein particle (RNP) complex (nucleocapsid protein + RNA bonded together) is formed. The RNP complex's helical structure creates a suitable RNA conformation for replication and transcription of the viral RNA.

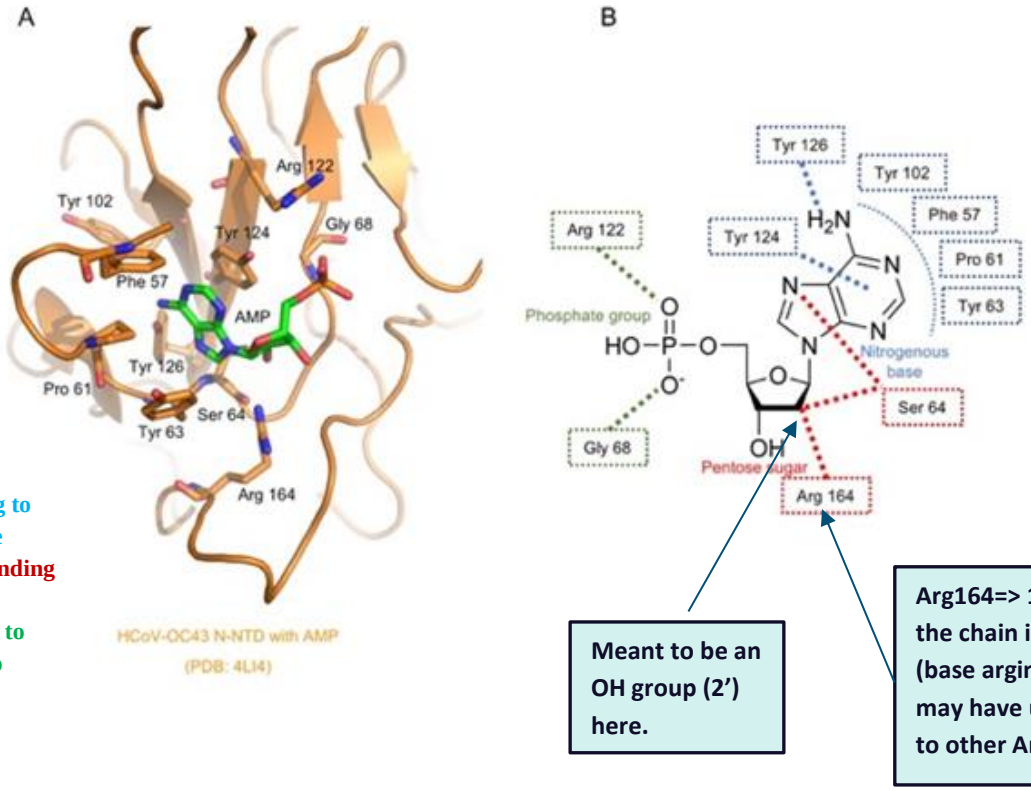
Although several CoV N-NTD structures have been identified, no RNA binding mechanisms have been described except for the mechanism of HCoV-OC43 (a CoV typically causing mild cold symptoms). The surface characteristics of N-NTD of SARS-CoV-2 vs HCoV-OC43 are distinct, but the HCoV-OC43 binding mechanism can be used to infer the mechanism for SARS-CoV-2. RNA contains several different nucleotides e.g. adenosine monophosphate (AMP)/uridine monophosphate (UMP)/cytosine monophosphate (CMP)/guanosine monophosphate (GMP). Previous studies have shown that HCoV-OC43 N-NTD contained AMP/UMP/CMP/GMP binding sites alongside the middle two β-strands of its β-sheets core (orange arrow = β-strand) shown in figure A in the diagram below[19].

[17] De Haan C.A., Rottier P.J. Molecular interactions in the assembly of coronaviruses. Adv. Virus Res. 2005;64:165–230.

[18] K.S. Saikatendu, J.S. Joseph, V. Subramanian, B.W. Neuman, M.J. Buchmeier, R.C. Stevens, et al., Ribonucleocapsid formation of severe acute respiratory syndrome coronavirus through molecular action of the N-terminal domain of N protein, J Virol, 81 (2007)

[19] S.Y. Lin, C.L. Liu, Y.M. Chang, J.C. Zhao, S. Perlman, M.H. Hou
Structural basis for the identification of the N-terminal domain of coronavirus nucleocapsid protein as an antiviral target, J Med Chem, 57 (2014)

Figure S4



Key:

Residues binding to nitrogenous base

pentose sugar binding residues

residues binding to phosphate group

A) 3D representation of N-protein showing the ribonucleotide (AMP) binding site in the centre.

B) Diagram showing where the amino acids on the N protein bind to the ribonucleotide.

This picture is linked to a website with a 3D N-NTD diagram showing the nitrogenous base and ligand interactions with the AA residues. https://www.rcsb.org/structure/

The binding mechanism for HCoV-OC43 shown in figure S4 above shows that;

- Arg122 and Gly68 were bound to phosphate groups via ionic interactions.
- Ser64 and Arg164 interacted (intermolecular forces and covalent bonds) with the ribose 2′-hydroxyl group.
- The amino acid residues listed in blue in figure S4 interacted with the nitrogenous base. They have hydrophobic R groups and are found in the 'hydrophobic pocket', out of these residues the base mainly interacted with Tyr124 (this residue contains R group(s) with aromatic rings) via π–π offset stacking forces (non-covalent forces arising from interaction from an aromatic ring on the base with a positive electrostatic potential i.e. electron deficient with an aromatic ring with a negative electrostatic potential i.e. with excess electrons forming an offset stack).

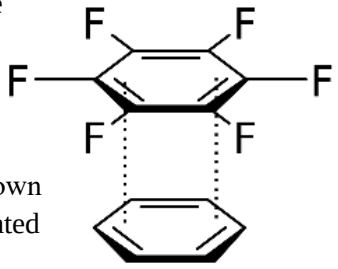
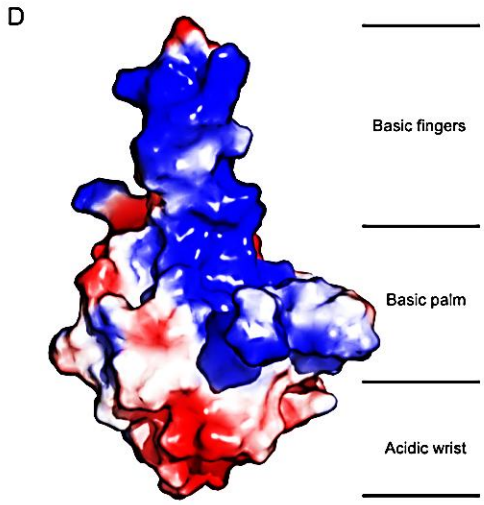
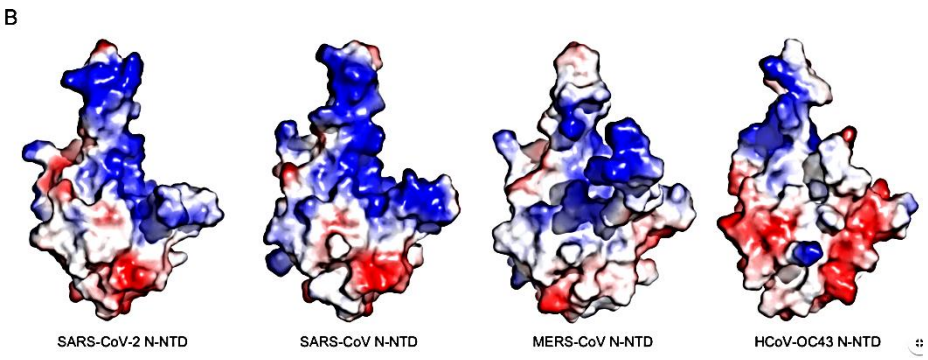


Figure 5 Offset stacking e.g. electron-rich benzene ring interacts with electron-poor hexafluorobenzene

The folded shape of the SARS-CoV-2 N-NTD (alternative 3D model to figure S4 and figure S3) shares common features with other coronaviruses. They all resemble a hand with basic 'fingers' that extend outwards, a hydrophobic 'palm' and an acidic 'wrist', shown in figure D below[20]. The amino acid residues mentioned above (and in figure S4) are located in the coloured regions of the diagram below, depending on the properties of their R groups e.g. amino acid residues with basic R groups will be found in the blue regions i.e. basic fingers/palm.



Right-hand shape structure of N-NTD



Basic = polypeptides with AA residues containing R groups that can accept H+.

Acidic = polypeptides with AA residues containing R groups that can donate H+.

Blue => Area of positive electrostatic potential. i.e. electron poor
Red => Area of negative electrostatic potential. i.e. electron rich
White => 0 electrostatic potential (non-polar hydrophobic parts i.e. hydrophobic pocket).

It is proposed that this ribonucleotide binding mechanism is essential for all coronavirus N proteins, including SARS-CoV-2, which is important in development of drugs which target the N-NTD and prevent the formation of the RNP complex, and hence interrupt replication and transcription of viral RNA.

[20] Viruses. 2014 Aug; 6(8): 2991–3018.Published online 2014 Aug 7

Figure 5- Why is the melting point of hexafluorobenzene in a 1:1 mixture with benzene so much higher than the individual melting points of about 5C? Socratic Q&A, Ernest Z., Ernest Z.

Membrane Protein

M protein is the most abundant viral protein[21], Recent studies suggest M protein exists as a dimer (two protein monomers), allowing it to have two different conformations. One conformation is used to promote membrane curvature, and the other to bind to the nucleocapsid (N) protein[22]. The exact functions of SARS-CoV-2's M is currently unknown, but it is understood that it can interact with all viral structural proteins (S, E, N), indicating its key role in virion assembly[23].

Envelope Protein

The envelope (E) protein is the smallest polyprotein in the SARS-Cov-2 structure. It has important roles in the production and maturation of the virus [24]. Some studies suggest depletion of E protein from the viral genome leads to significant reductions in viral growth and virion formation, suggesting its importance in viral assembly[25]. The E protein appears to induce membrane curvature in the Endoplasmic Reticulum-Golgi intermediate compartment (ERGIC) shown in the diagram below, which promotes membrane scission of the virion particle and hence its release from the compartment where it can then exit the cell via exocytosis[26].

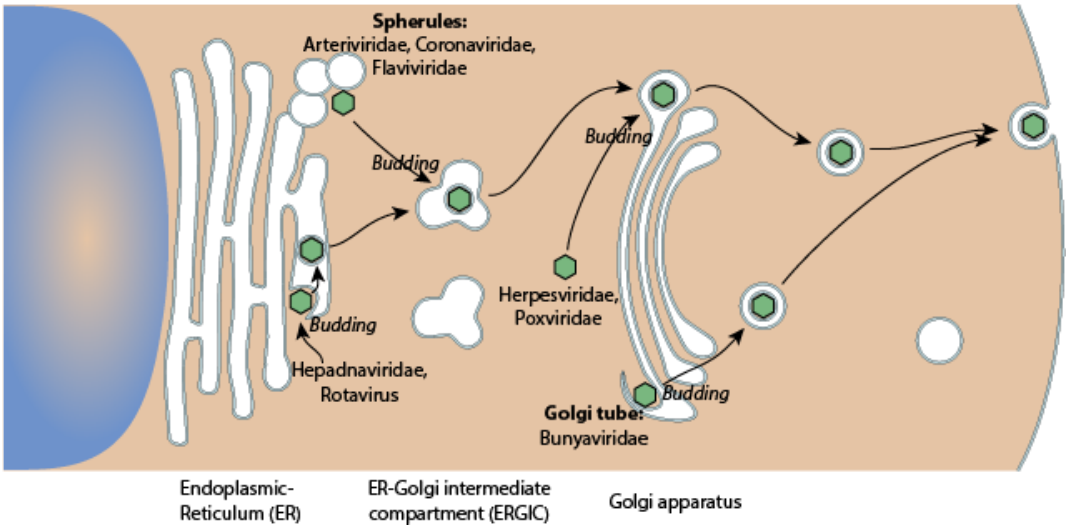


Figure 6 Virus exocytosis

[21] Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 2011

[22] Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, Droese B, Klaus JP, Makino S, Sawicki SG, Siddell SG, Stamou DG, Wilson IA, Kuhn P, Buchmeier MJ, J Struct Biol. 2011

[23] Masters PS. The molecular biology of coronaviruses. Adv Virus Res. 2006

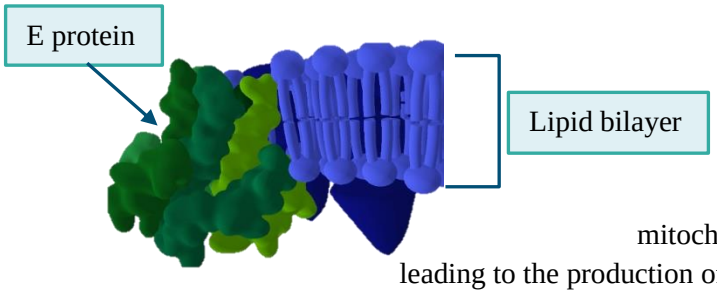
[24] 12. Schoeman D., Fielding B.C. Coronavirus envelope protein: current knowledge. Virol J. 2019;16:69.

[25] DeDiego, M. L., E. Alvarez, F. Almazan, M. T. Rejas, E. Lamirande, A. Roberts, W. J. Shieh, S. R. Zaki, K. Subbarao, and L. Enjuanes. 2007. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. J. Virol.

[26] Vennema H, Godeke GJ, Rossen JW et al. Nucleocapsid-independent assembly of coronavirus-like particles by co-expression of viral envelope protein genes. EMBO J. 15(8), 2020–2028 (1996)

Figure 6- Virus budding by cellular exocytosis, viralzone, https://viralzone.expasy.org/5899

The SARS-CoV-2 E protein has also been found to function as a viral ion-channel (viroporins). The SARS-CoV-2 viroporins exist as homopentamers (5 protein subunits). Each subunit is ~50-120 amino acids long and has at least one transmembrane domain (TMD), i.e. protein regions that span the lipid bilayer as shown below and in figure S1. The TMD forms a pore in the lipid bilayer, which acts as an ion channel.



The SARS-CoV-2 E protein ion channel has been found embedded in ERGIC/golgi membranes facilitating Ca2+ transport to the host's mitochondria. It is proposed that excessive Ca2+ released from ER causes mitochondrial Ca2+ overload and mitochondrial damage, leading to the production of mtROS (reactive oxygen species i.e. reactive chemical species containing oxygen, found in the mitochondria.) mtROS production triggers the activation of an inflammasome (protein complexes responsible for activation of inflammatory responses), called NLPR3. The activation is shown in the diagram below from ROS -> NLPR3 step onwards.

PMID: 2445029

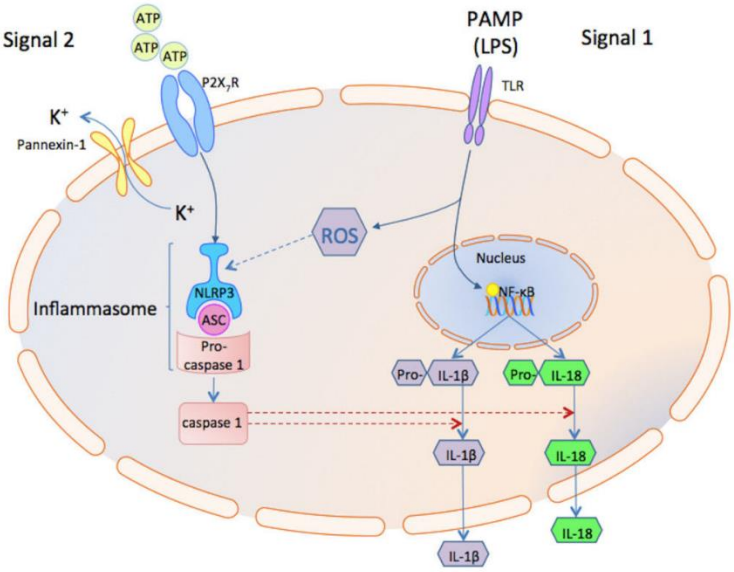


Figure 7 oligomerization of NLRP3

Activated NLPR3 recruits apoptosis-associated-speck like proteins (ASC) and pro-caspase 1 leading to caspase 1 activation. Activated caspase 1 cleaves cytokine precursors pro-IL-1β and pro-IL-18, forming mature IL-1β and IL-18, and promotes their secretion. IL-18 encourages the production of IFN- γ [27], which promotes transcription of interferon-stimulated genes (discussed further in the immune response section). IL-1β follows a signal transduction pathway that results in a strong proinflammatory signal (e.g. pro-inflammatory cytokine release). Therefore, these two IFNs together, result in an excess proliferation of pro-inflammatory cytokines, which is likely to be a major contribution to the 'cytokine storm' identified ~7-10 days during severe COVID-19 cases[28]. The antioxidant effects (further discussion in vitamin C section) of Vitamin C may inhibit ROS production and hence regulate NLRP3 activation, which may be a potential treatment for reducing the cytokine storm.

[27] Nieto-Torres, J.L., et al., Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology, 2015.

[28] Jimenez-Guardeño, J.M., et al., The PDZ-Binding Motif of Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Is a Determinant of Viral Pathogenesis. PLOS Pathogens, 2014.

Figure 7- INFLAMMASOME (NLPR3), GBS Leiden, http://gbsleiden.com/inflammasome-nlpr3/

Entry Mechanisms

It is likely that SARS-CoV-2 enters cells either through an endocytic pathway or through direct fusion with the plasma membrane. SARS-CoV-2 can bind to ACE2 receptors but also sialic acid residues on gangliosides (glycosphingolipids on the lipid raft) e.g. GM1[29]. The N-terminal domain (free NH2 group(s) at the end of a peptide) of the SARS-CoV-2 S protein can bond with the oligosaccharide (3-10 sugar/monosaccharide monomers) portion of the ganglioside [30]aka the ganglioside binding domain (GBD) shown in the diagram below.

[31]

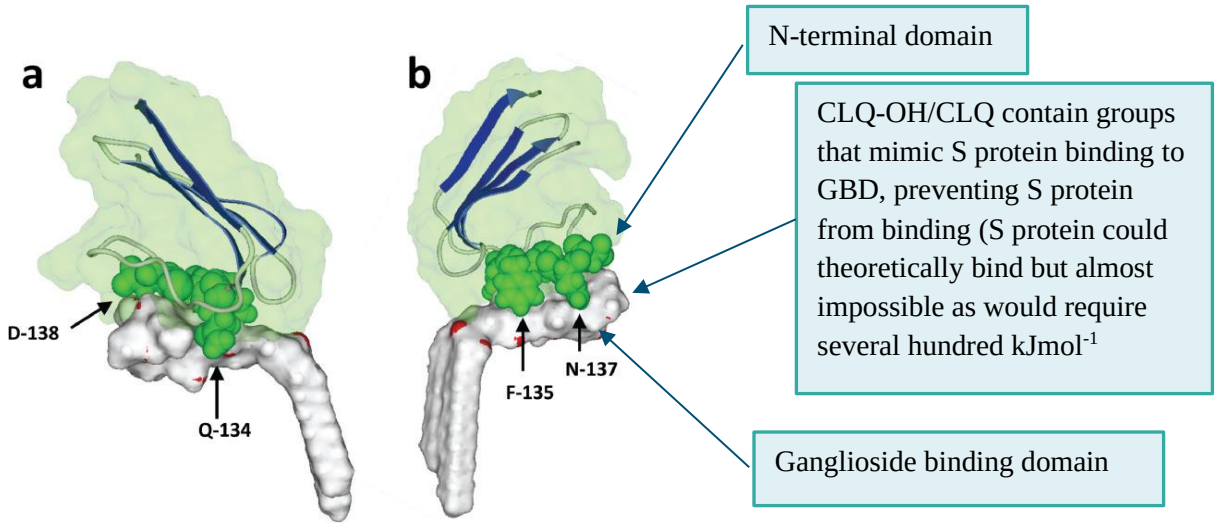


Figure 8 Two symmetric views of N-terminal domain binding to GBD

Once the S protein is bound, spike protein-membrane fusion is initiated by the TMPRSS2 cleaving the S1/S2 boundary of S protein causing a conformational change from a pre-fusion structure to a post-fusion structure (coiled fusion peptide), releasing free energy. The fusion of two lipid bilayers is thermodynamically favourable[32], as the fused membrane is more stable than the original membrane. However, it is not spontaneous kinetically (has a high Ea so the reaction is so slow it doesn't 'go'), so the free energy released during the conformational change of the S protein is used to increase the kinetic energy of the S protein, and hence overcome the activation enthalpy. Direct fusion transports ACE2 with bound S protein into the cell[33], releasing the virion contents. SARS-CoV-2 may also be able to enter through caveolin-dependent endocytosis, where the binding of S protein to ACE2 induces an invagination in the cell membrane forming a caveolae (caveolar vesicle). Release of the caveolar vesicle is mediated by GTPase dynamin II, which snips the vesicle free from the membrane (membrane scission). The released vesicle then fuses which then fuses with an early endosome or caveosome where the endocytic pathway (exact mechanism for SARS-CoV-2 is unknown) continues and the

[29] Matrosovich M., Herrler G., Klenk H.D. Sialic acid receptors of viruses. Top Curr Chem. 2015;367:1–28

[30] Fantini, J., Di Scala, C., Chahinian, H. and Yahi, N., 2020. Structural And Molecular Modelling Studies Reveal A New Mechanism Of Action Of Chloroquine And Hydroxychloroquine Against SARS-Cov-2 Infection. Int J Antimicrob Agents. 2020 Apr 3

[31] Matrosovich M., Herrler G., Klenk H.D. Sialic acid receptors of viruses. Top Curr Chem. 2015

[32] Chernomordik LV, Kozlov MM. Protein-lipid interplay in fusion and fission of biological membranes. Annu. Rev. Biochem. 2003;72:175–207. doi: 10.1146/annurev.biochem

[33] Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005

virion contents are released. The exact mechanism for cell entry of SARS-CoV-2 could depend on the type of cell as there have been different results found for different types of cells[34][35].

Mechanism of action *e.g. what happens to RNA and how are virions made?*

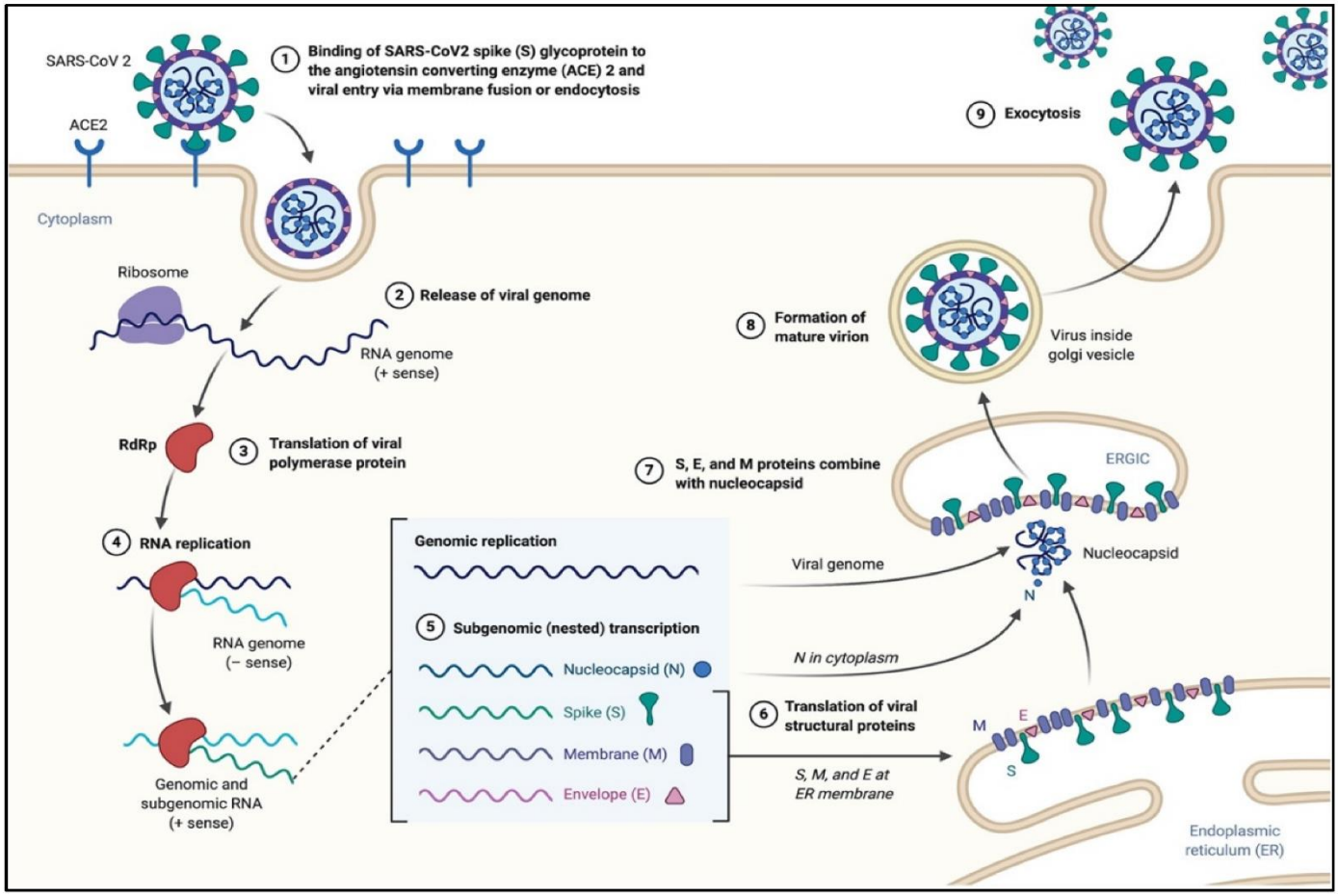


Figure 9 Life cycle of SARS-CoV-2 in host cell

After the virion contents are released inside the cell, the virus utilises replication processes within the cell to synthesise viral proteins. The mechanism of RNA replication revolves around the replicase gene, which contains code for non-structural proteins (NSPs), which are associated with RNA synthesis, and structural proteins which are associated with virion assembly. The replicase gene codes for two large open reading frames (sections of a gene aka ORFs) rep1a and rep1ab, which express polyproteins pp1a and pp1ab respectively. Let rep1a = frame 1 and rep1ab = frame 2 as shown in the diagram below. Translation along frame 1 would occur

[34] Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G. et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. Cell Res. 2008

[35] Inoue Y, Tanaka N, Tanaka Y, Inoue S, Morita K, Zhuang M. et al. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. J Virol. 2007

Figure 9- The Human Coronavirus Disease COVID-19: Its Origin, Characteristics, and Insights into Potential Drugs and Its Mechanisms, Lo'ai Alanagreh, ,Foad Alzoughool andManar Atoum, Received: 31 March 2020

until a stop-codon is reached, this would produce pp1a but not pp1ab. Pp1ab is produced by translation of frame 2 which can only be reached if a frameshift occurs from frame 1 to frame 2. SARS-Cov-2 uses a slippery sequence and an RNA pseudoknot to do this. Normally the ribosome fully unwinds the pseudoknot structure, translating until it reaches the frame 1 stop codon, producing pp1a. However, the ribosome may be blocked by the pseudoknot forcing it to pause on the slippery sequence which shifts the reading frame one nucleotide backwards (a frameshift of -1) extending translation into frame 2 shown below.

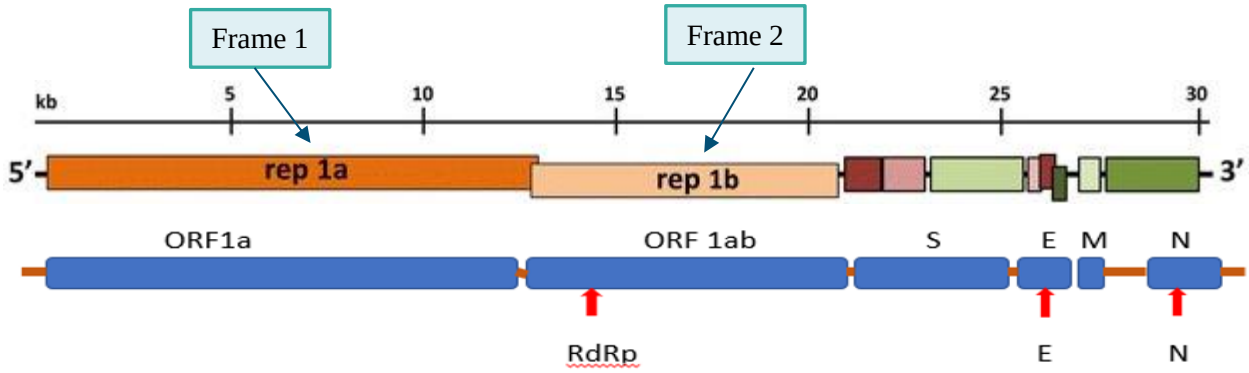


Figure 10 rep1a and rep1ab

This results in the translation for polyprotein pp1ab that wouldn't happen otherwise.

It is hypothesised that (some) coronaviruses utilise ribosomal frameshifting to control the ratio of rep1a proteins: rep1b proteins or delay the production of rep1b proteins until proteins produced from rep1a have created a suitable environment for RNA replication.

The polyproteins pp1a and pp1ab contain non-structural proteins. These polyproteins are cleaved by proteases forming NSPs with specific essential functions e.g. some may form the replicase-transcriptase complex (RTC) which is responsible for RNA replication and creating a suitable environment for RNA synthesis, and some may form important enzymes involved in RNA replication like RNA-dependent RNA polymerase (RdRp) and RNA Helicase. Other polyproteins on different reading frames are cleaved to form structural proteins e.g. membrane (M), spike (S), envelope (E), nucleocapsid (N) proteins. These structural proteins are translated, encapsulated by N protein, and inserted into the endoplasmic reticulum (ER) where they bud into membranes of the endoplasmic reticulum-golgi intermediate compartment (ERGIC)[36][37], forming mature virions (capsules containing viral proteins that are ready to infect). Mature virions are then transported to the cell surface membrane where they exit via exocytosis.

In several coronaviruses, S protein that does not get assembled into virions transits to the cell surface where it mediates cell–cell fusion between infected cells and adjacent, uninfected cells. This leads to the formation of giant, multinucleated cells (Syncytia) which allows the virus to spread within an infected organism without being detected or neutralized by virus-specific antibodies.

[36] Tooze J, Tooze S, Warren G. Replication of coronavirus MHV-A59 in sac-cells: determination of the first site of budding of progeny virions. Eur J Cell Biol. 1984

[37] Krijnse-Locker J, Ericsson M, Rottier PJM, et al. Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. J Cell Biol. 1994

Figure 10- Coronaviruses: an overview of their replication and pathogenesis., Fehr AR, Perlman S, Methods in Molecular Biology (Clifton, N.J.), 01 Jan 2015

Immune response

Based on the genomic sequence comparison, SARS-CoV2 shares overall genomic similarity with SARS-CoV and MERS-CoV, approximately 79% and 50%. Therefore, careful sequence comparison of each gene region gives an idea of the nature of the immunology of SARS-CoV-2, and the biology of the virus should also be similar. This suggests that viral replication of SARS-CoV-2 results in suppression of type I interferon (type 1 IFN) and an influx of neutrophils and macrophage cells which are the major sources of pro-inflammatory chemicals called cytokines.[38]

1. SARS-CoV-2 suppresses type I IFN. This causes the loss of viral control in an early phase of infection.

2. This indicates that the natural immune response is a critical factor for disease outcome (i.e. Damage to the body, chance of death or chance of recovery)

3. Suppression of type I IFN also triggers an influx of pro-inflammatory cytokines

4. The inflammation and the pneumonia of lungs could be caused by these "cytokine storms"

5. T-cells: TH17 and TH1 cells can cause inflammation and autoimmune disease[39]. In SARS-CoV-2 infection, they are activated and contribute to amplifying the inflammatory responses.

6. B cells/plasma cells produce SARS-CoV-2 specific complementary antibodies that bind on antigen receptors and neutralize the virus.

*(*all the information above is referenced on the paper published by Asian Pacific Journal of Allergy and Immunology. Not all the information is accurate as the immunology of SARS-CoV-2 is not yet fully studied. This is just a brief estimate of the immune response)*

Innate immunity

The effector components of innate immunity include epithelial barriers, phagocytes, and natural killer (NK) cells, as well as cytokines and the complement system, however, as shown in the diagram below, natural killer cells don't seem to play a significant part in the innate response to SARS-CoV-2 compared to B cells and phagocytes.

[38] Eakachai P, Chutitorn K,Tanapat P, Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic, Asian Pac J Allergy Immunol 2020;38:1-9 DOI 10.12932/AP-200220-0772

[39] Ann N Y Acad Sci, Th1 and Th17 cells, US National Library of Medicine National Institutes of Health Search database, 2010 Jan

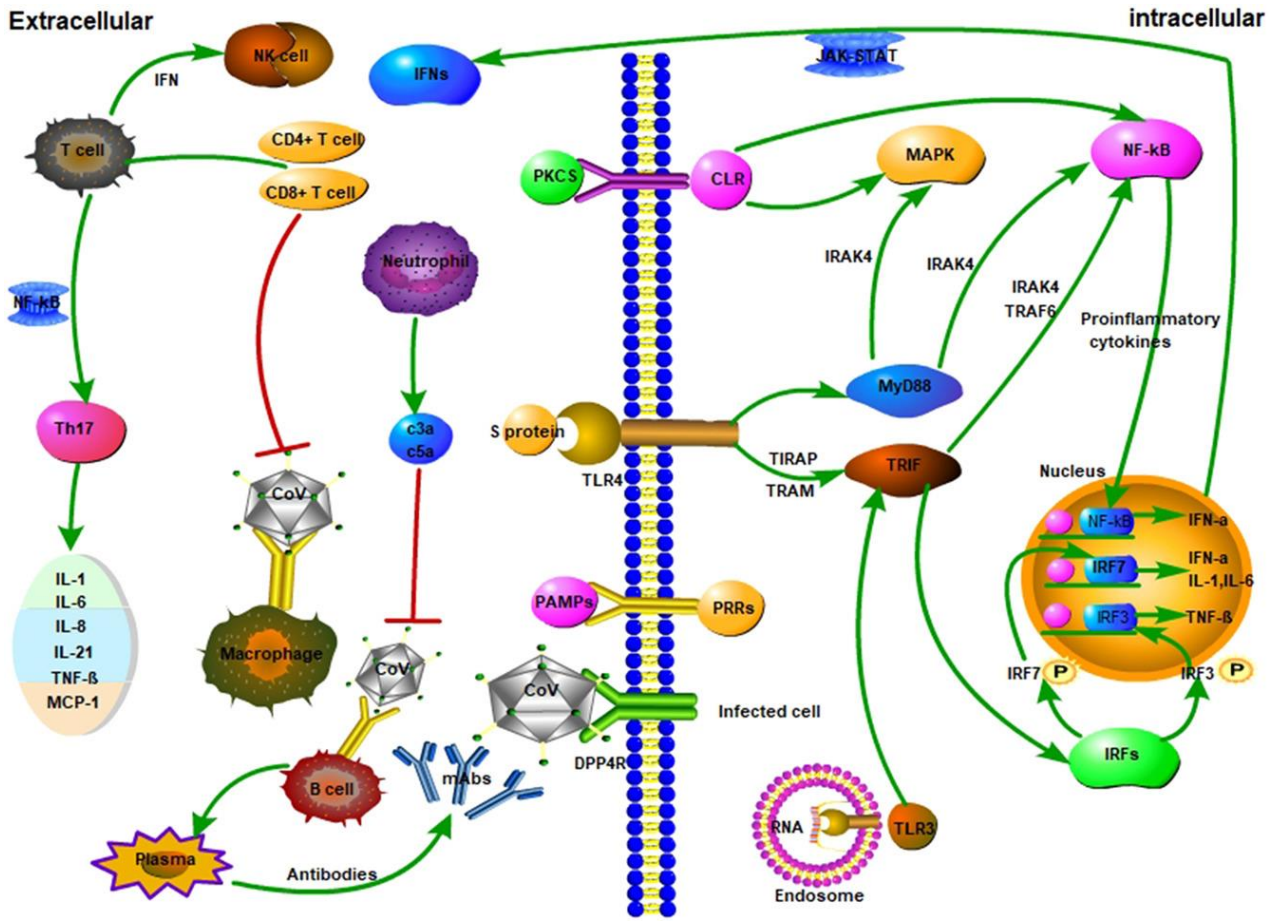


Figure 11 Innate immune response mechanisms of coronavirus

NK cells

Research shows that during infection, CD8+ T cells and exhausted NK cells (NK cells that express an altered phenotype and poor function during infection due to the presence of suppressive cytokines released by the virus) express higher levels of NKG2A receptor which binds to MHC Class I complex receptor derivative HLA-E expressed on target cells, which causes an inhibition of the NK cell, suppressing cytotoxic activity and hence not destroying the target cell[4041]. This could be the reason why NK cells don't play a significant role in the innate response, and potentially why levels of immune cells e.g. neutrophils can spiral out of control on recruitment by chemokines in a cytokine storm, causing severe inflammation.

Lectin Pathway

There is evidence to suggest that the lectin pathway of the complement cascade is utilised in the innate response to SARS-CoV-2. The lectin pathway is initiated when mannose-binding lectin (a recognition molecule aka MBL) binds to pathogen-associated molecular patterns (e.g. PAMPs) expressed on the surface of the virus. MBL then forms a complex with dimers MBL-associated serine proteases (MASPs) MASP1 and MASP2. The

Figure 11- Coronavirus infections and immune responses, Geng Li Yaohua Fan Yanni Lai Tiantian Han Zonghui Li Peiwen Zhou Pan Pan Wenbiao Wang Dingwen Hu Xiaohong Liu Qiwei Zhang Jianguo Wu, First published:25 January 2020

41 Qin, C. et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin. Infect. Dis. (2020).

formation of the complex activates MASP1 automatically activates MASP2 leading to cleavage of two complement proteins, initiating a cascade which produces complement proteins e.g. (C3b) that tag antigens/pathogens for opsonisation (opsonisation leads to enhanced phagocytosis) and terminal complement protein complex which is inserted into the cell membrane which alters the cell membrane structure leading to cell death[42].

Specific/Cell mediated immunity

In respiratory viruses like SARS-CoV-2, dendritic cells (DCs) are the main mediators of an immune response and hence the inflammatory e.g. pneumonic symptoms experienced. Immature DCs express pattern recognition receptors (PPR) specific for Pathogen-associated molecular pattern molecules (PAMPs). An immature DC will bind to PAMPs on infected cells(antigen presenting cells) triggering the differentiation from an immature DC to a mature DC causing the increased acidification of the lysosomes to optimise/increase up-regulation (production) of costimulatory molecules, antigen processing, and organisation of MHC Class II from late endosomes to the plasma membrane[43][44]. Before/After maturation the DC synthesises MHC, Class II molecules bonded to invariant chain protein (li shown in the diagram below) in the endoplasmic reticulum. The li-MHC class II complex is transported from the ER to the Golgi then to the plasma membrane where they are packaged into an early endosome by clathrin-mediated endocytosis.

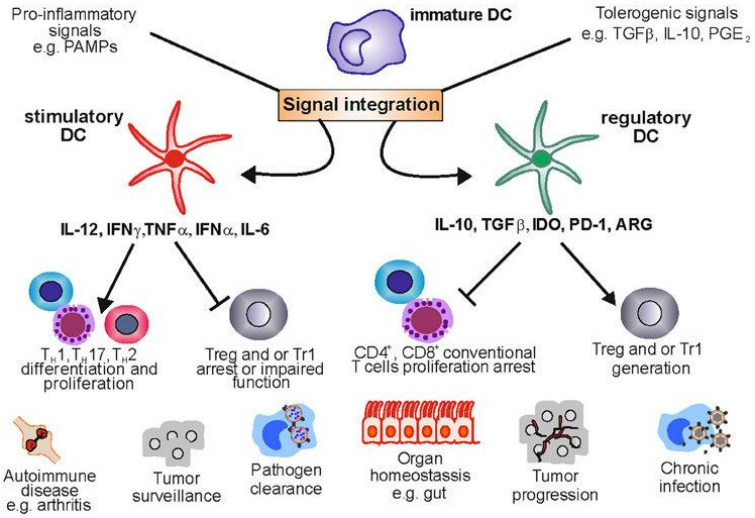


Figure 12 Dendritic cell signal integration

The mature dendritic cells in question are likely to be stimulatory dendritic cells due to the stimulation being caused by the recognition of PAMPs on APC and the release of specific cytokines related to pathogen clearance as shown in the diagram above.

The invariant protein (li) contains targeting sequences that direct the li-MHC Class II complex to the endosomal-lysosomal antigen-processing compartments where antigenic proteins/peptides (antigens) are found (that have also been taken in by clathrin-mediated endocytosis once the DC bound to an infected antigen presenting cell). li is then proteolytically degraded and dissociates from the complex allowing antigens to bind to MHC Class II forming the MHC Class II complex that is transported to the plasma membrane and expressed on the surface. Activated dendritic cells migrate to the draining lymph nodes (DLN) where naïve CD4+ T cells bind to the complementary MHC class II receptors, leading to activation. Activated T cells migrate to the area of

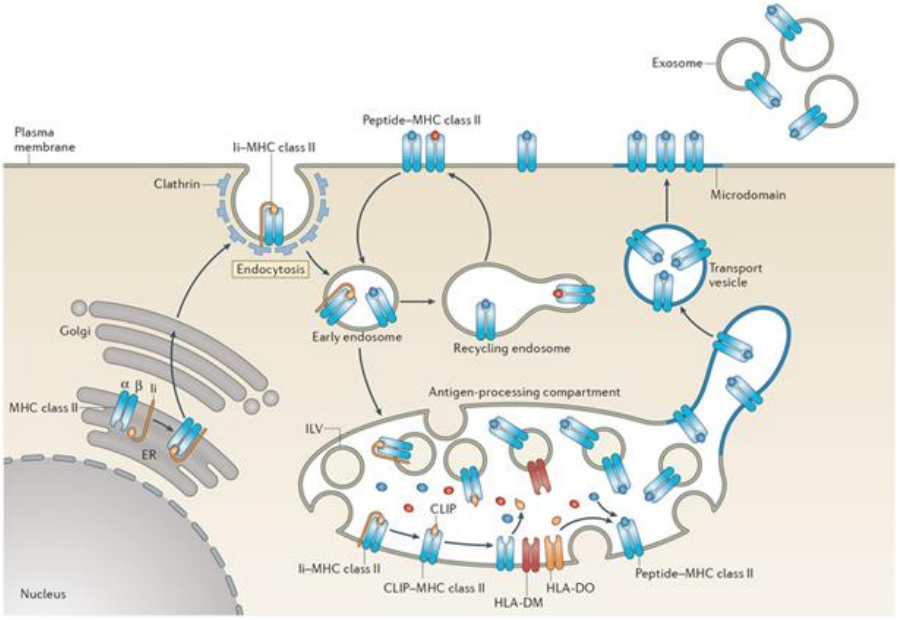
[42] osbrink M, Niculescu F, Rus H. The role of c5b-9 terminal complement complex in activation of the cell cycle and transcription. Immunol Res (2005)

[43] Pierre P, et al. (1997) Developmental regulation of MHC class II transport in mouse dendritic cells. Nature 388:787–792.

[44] Cella M, Engering A, Pinet V, Pieters J, Lanzavecchia A (1997) Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. Nature 388:782–787.

Figure 12- Regulatory dendritic cells: There is more than just immune activation, Susanne Viktoria Schmidt, Andrea Nino, Joachim L. Schultze, Sep 2012

infection where they produce specific anti-viral cytokines (IFN-γ, TNF-α, IL-2, IL-5, IL-6), chemokines (CXCL-9, 10 and 11) and cytotoxic molecules (perforin and granzyme B)[45].



Activated CD4+ T cells can also bind to B cell receptors (CD40) and then release cytokines that encourage the proliferation of and differentiation of B cells into plasma cells which have several roles in immunity shown in the diagram below. T-cell-independent B cell activation can occur via direct receptor interaction between B cells and antigens on the surface of the pathogen, however, the differentiation of these B cells forms plasma blasts which release antibodies of weaker affinity and a shorter lifespan than those of T-cell dependent plasma cells, so are less significant to the immune response.

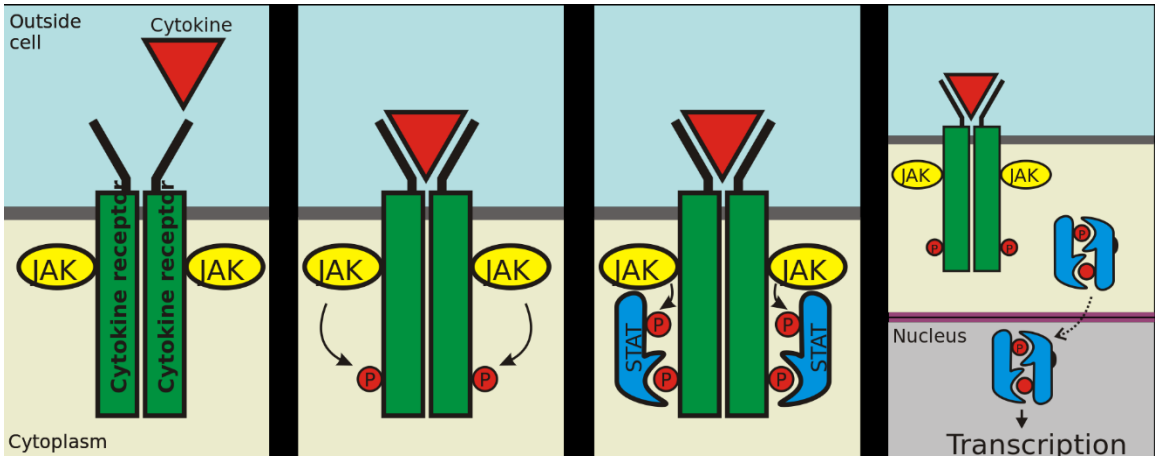


Figure 13 Interferons and interleukins mainly signal through the JAK-STAT pathway.

JAK-STAT signalling involves three major proteins: cell-surface receptors, Janus kinases (JAKs), and signal transducer and activator of transcription proteins (STATs). Once a ligand (red triangle) binds to the receptor, JAKs add phosphates (red circles) to the receptor. Two transcription (STATs) proteins then bind to the phosphates, and then the STATs are phosphorylated by JAKs to form a dimer (two protein complex). The dimer enters the nucleus, binds to DNA, and causes transcription of interferon-stimulated genes (ISGs) in the target cell e.g. IFITM3 protein which is suggested to inhibit fusion of the virus-membrane or reside in endocytic vesicles where it increases the rate of trafficking of viral vesicles to the lysosomes for degradation[46]. Other

[45] Wherry EJ, Ahmed R. Memory CD8 T-cell differentiation during viral infection. J Virol. 2004

[46] Spence JS, He R, Hoffmann HH, Das T, Thinon E, et al. 2019. IFITM3 directly engages and shuttles incoming virus particles to lysosomes. Nat. Chem

Figure 13- Key elements of the JAK-STAT pathway, Peter Znamenkiy, Created: 1 July 2006

proteins from ISGs may have different functions like bonding to the nucleocapsid protein and mediating proteasome-dependent destruction of the viral core, and apoptotic mechanisms[47].

If SARS-CoV-2 is not sufficiently cleared by the early acute phase/innate immune response COVID-19 symptoms might reach severe levels. Most patients with severe COVID-19 around the 7-10 day mark, show high serum levels of pro-inflammatory cytokines including IL-6 and IL-1β, as well as IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α (also known as CCL3) and TNF- α, whilst showing decreased levels of CD8+ and IFN-γ–expressing CD4+ T cells, characterised as cytokine storm[48].

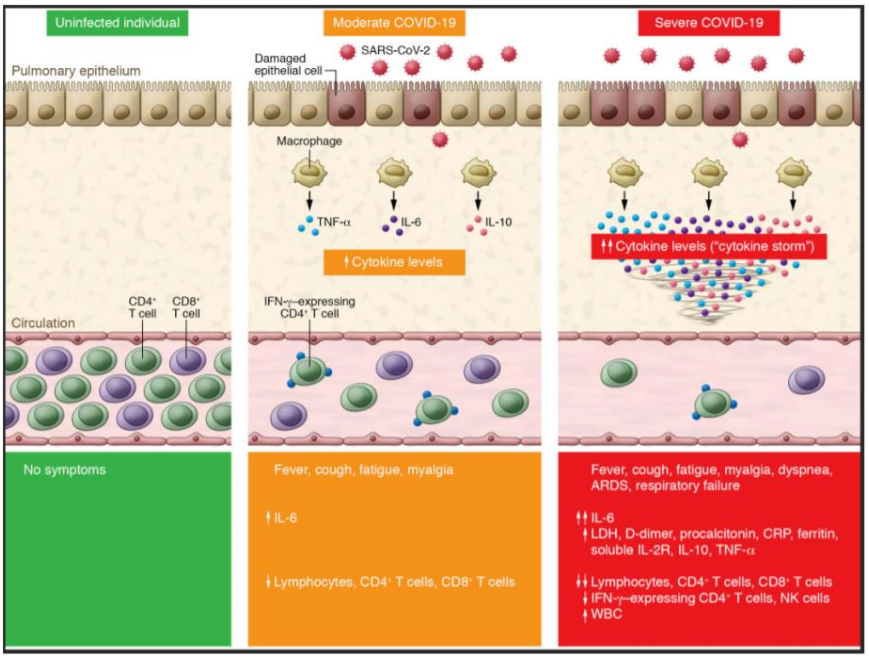


Figure 14 Cytokine storm and T cell lymphopenia is associated with COVID-19 disease

The cytokine storm showed in the diagram above, occurs when large numbers of white blood cells (WBC) e.g. dendritic cells and T cells, are activated and release pro-inflammatory cytokines which in turn activates more white blood cells creating a positive feedback loop of pathogenic inflammation leading to hyperinflammation[49]. This hyper inflammation results in inflammatory lymphocytic and monocytic infiltration of the lungs and heart causing acute respiratory distress syndrome (ARDS) and increased risk of cardiac failure[50].

[47] Kutluay SB, Perez-Caballero D, Bieniasz PD. 2013. Fates of retroviral core components during unrestricted and TRIM5-restricted infection. PLOS Pathog. 9: e100321468. Ganser-Pornillos BK, Chandrasekaran V, Pornillos O, Sodroski JG, Sundquist WI, Yeager M. 2011. Hexagonal assembly of a restricting TRIM5α protein. PNAS 108: 534–3969. Wu X, Anderson JL, Campbell EM, Joseph AM, Hope TJ. 2006. Proteasome inhibitors uncouple rhesus TRIM5α restriction of HIV-1 reverse transcription and infection.

[48] Qin, C. et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin. Infect. Dis. https://doi.org/10.1093/cid/ciaa248 (2020).

49 Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL (July 2014). "Current concepts in the diagnosis and management of cytokine release syndrome". Blood. 124 (2): 188–95. doi:10.1182/blood-2014-05-552729.

[50] Zhang C, et al. "The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality". International Journal of Antimicrobial Agents.

Figure 14- SARS-CoV-2: A Storm is Raging, Savannah F. Pedersen, Ya-Chi Ho, J Clin Invest. 2020

Transmission Mechanisms

There are 3 main ways of COVID-19 transmission:

- Close contact and respiratory droplets
- Airborne Transmission
- Objects and Surfaces

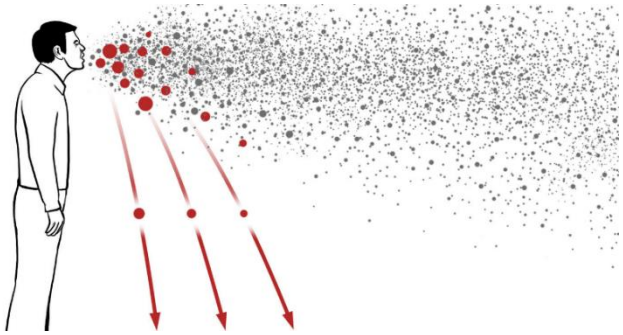


Figure 15 Large droplets (red) falls to surfaces and small droplets (grey) floats in the air

Close contact and respiratory droplets

Virus proteins are found in the respiratory system, and the virus can also build up in droplets/mucus. As the infected person coughs, sneezes, or talks, droplets can land on the mouth or nose of people who are nearby, and as the person breathes in, the virus can be inhaled to the respiratory system[51]. Small viral-laden aerosolized droplets can remain in the air and travel over a meter, making it easy for the virus to transmit to others[52].

Airborne Transmission

Airborne Transmission is defined as the spread of an infectious agent caused by the dissemination of droplet nuclei that remain infectious when suspended in air over long distances and time[53]. Meaning droplet nuclei containing SARS-CoV-2 can suspend in the air like water droplets. Researchers found out that the airborne transmission distance of SARS-CoV-2 is up to 4 meters[54]. According to the study published in The New England Journal of Medicine on March 17, Virus can remain viable "in aerosols up to 3 hours", This makes places like hospital wards and restaurants hostile and vulnerable, explains why this virus seems to spread so easily in places with poor ventilation.

Objects and Surfaces

As the infected person releases droplets, large droplets can land on hard surfaces like door handles. When another person has contact with contaminated surfaces and touches their face, especially the eyes, nose, or mouth, the virus could transmit. The SARS-CoV-2 virus has a surprisingly long lifespan without the presence of the host. On cardboard, the virus can survive for a whole day, and on plastic and stainless steel, the virus can even survive for 2 to 3 days[55].

[51] How COVID-19 spreads, US Centres for Disease Control and Prevention (CDC), retrieved 02/05/2020

[52] Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations, WHO, Published on 29 March 2020, https://www.who.int/news-room/commentaries/detail/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations

[53] Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care WHO Guidelines, 2014

[54] Emerging Infectious Diseases, Volume 26, Number 7—July 2020, Centres of disease control and prevention

Figure 15- MICHAELEEN DOUCLEFF, Ebola In the Air: What Science Says About How the Virus Spreads, December 1, 2014, Adam Cole/NPR

[55] Emerging Infectious Diseases, Volume 26, Number 7—July 2020, Centres of disease control and prevention

Viral Exit

After the virus protein is synthesised, Virions are made. Virions are the vector stage of the virus, where they leave the cell and infect other healthy cells. Although currently there is not enough research on viral shedding of SARS-CoV-2, SARS-CoV-2 can be released in 3 possible ways through virus shedding:

- Via exocytosis
- Via Budding
- Via apoptosis

Via exocytosis

After virion is synthesized, it's wrapped around by a vesicle when it leaves the Golgi apparatus. The virus vesicle is then transported towards the cell membrane and leaves the cell[56]. The virus is released to extracellular space, and it will travel around the body and infect other healthy cells.

Via Budding

Virus budding is the exocytosis of the virus along with borrowing the host cell's cell membrane. To initiate budding, virus nucleocapsid interacts with a certain part of the host cell membrane. The viral envelope protein binds with the host cell membrane. To successfully bud from the host cell, the nucleocapsid of the virus must bind with the cytoplasmic tails of envelope proteins[57]. Virus budding allows the virus to have a disguise and reduces the chance of macrophages detecting the virus. It also uses up the host cell membrane, slowly degenerating and cause cell lysis, releasing more virus to extracellular space[58].

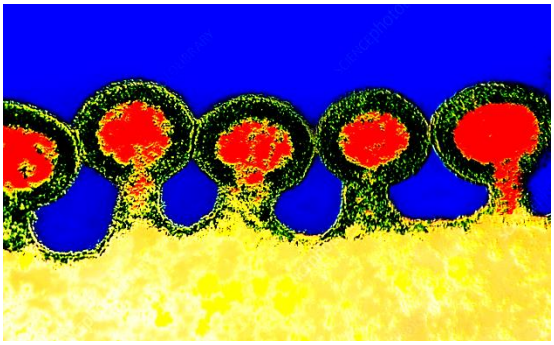


Figure 16 Coloured Trans- mission Electron Micrograph of HIV virus budding. The virus (red) is leaving the host cell and brings along the host cell membrane (dark green)

[56] Payne, Susan (2017). "Virus Interactions With the Cell". Viruses: 23-25. Retrieved 7 April 2020.

[57] Payne, Susan (2017). "Virus Interactions With the Cell". Viruses: 23-25. Retrieved 7 April 2020.

[58] Pornillos O, Garrus JE, Sundquist WI (December 2002). "Mechanisms of enveloped RNA virus budding". Trends in Cell Biology.

Via apoptosis

As viruses attack a host cell, the host cell is programmed to commit suicide to protect other healthy cells around it and delay virus spread. Normally, the host cell will chop up all the genetic material and be voluntarily engulfed by macrophages. Some viruses can take advantage of this being purposely engulfed by the macrophage, then infect it, thus using the macrophage to travel around the host body to infect other healthy cells[59]. But, only small amounts of ACE2 receptors are present on macrophage membranes, so it is still not clear if SARS-CoV-2 infects macrophages[60].

[59] Stewart SA, Poon B, Song JY, Chen IS (April 2000). "Human immunodeficiency virus type 1 vpr induces apoptosis through caspase activation". Journal of Virology.

[60] Eakachai P, Chutitorn K,Tanapat P, Immune responses in COVID-19 and potential vaccines:
Lessons learned from SARS and MERS epidemic, Asian Pac J Allergy Immunol 2020;38:1-9 DOI 10.12932/AP-200220-0772

Virus Sampling and Testing

SARS-CoV-2 is a virus that infects the respiratory tract and the intestines, as there are ACE2 receptors present in both places. So, when a person is infected, virus test samples are usually collected in those places using a nasopharyngeal swab, throat swab, sputum sample or stool sample[61]. Nasopharyngeal swab and throat swab testing are only accurate within the first week of infection, as the virus can travel down the respiratory tract to the lungs. By then, sputum sampling is used to test the lower respiratory tract by coughing or suction catheter[62]. Stool sampling is a collection of human faces, it is usually a test for children.

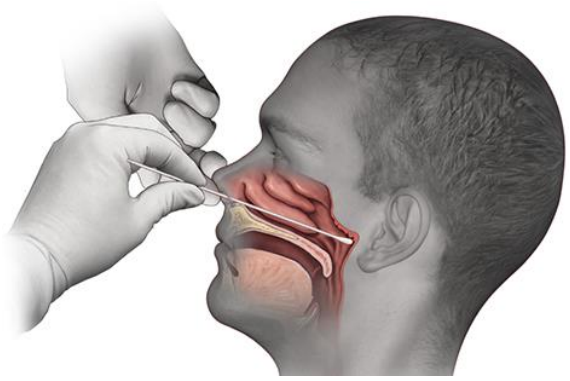


Figure 17 Obtaining a Nasopharyngeal Swab Specimen

RT-PCR

Reverse transcriptase-polymerase chain reaction (RT-PCR) is a rapid, highly sensitive and specific method to check the presence of a virus. RT-PCR test can be used to identify the presence of the virus by amplifying the virus RNA[63], results are usually available within a few hours to two days. RNA is first transcribed into cDNA (DNA synthesised from single-stranded RNA) which is then used as a template for the polymerase chain reaction (PCR). The polymerase chain reaction follows several steps:

1. Denaturation: Hydrogen bonds between complementary bases in DNA are broken by temperatures T>90 °C forming two single DNA strands.
2. Annealing: At 55-65°C primers can bind to complementary sequences on template DNA strands.
3. Extension: At ~72°C Taq polymerase extends primers on each DNA strand forming two double-stranded DNA molecules.

Each cycle produces 2n DNA molecules where n= number of cycles. Typically, PCR cycles repeat 35-45 times creating 235 DNA copies = 34,359,738,368 copies. Three genes of the SARS-CoV-2 including N, Orf1ab and E are targeted in the RT-PCR assay and primers and TaqMan probes are designed in the conserved region of the SARS- CoV-2 virus-specific genome region. These genes are then amplified and studied in detail or detected in virus testing kits[64].

Figure 18 RT-PCR machine

[61] "Real-Time RT-PCR Panel for Detection 2019-nCoV". Centers for Disease Control and Prevention. 29 January 2020

[62] Drosten, Christian (26 March 2020). "Coronavirus-Update Folge 22"

Figure 17- Coronavirus (COVID-19) testing: What you should know, UC Davis Health, Updated June 8, 2020

[63] Freeman WM, Walker SJ, Vrana KE (January 1999). "Quantitative RT-PCR: pitfalls and potential". BioTechniques.

Figure 18- New Real-Time PCR Systems Connect Researchers in the Cloud, scientific technology news, August 11, 2017

[64] QuantiVirus™ Real-Time PCR Coronavirus (SARS-CoV-2) Detection Test https://www.generon.co.uk/other-products-186/quantivirus-real-time-pcr-coronavirus-701000075.html

ELISA

ELISA (enzyme-linked immunosorbent assay) is another testing method for SARS-CoV-2. The test relies on the binding of complementary receptors to the virus antigen, producing a colour signal with an enzyme-substrate reaction if positive[65]. This is the Indirect ELISA test mechanism[6667]:

1. The SARS-CoV-2 virus or its antigen is buffered and coated evenly in a microtiter plate.
2. Non reacting protein is added to the microtiter plate to cover any plastic surfaces that are not coated with the virus antigen
3. A sample of the person's blood plasma is extracted to test the presence of the antibody for SARS-CoV-2
4. The blood sample is then added to the microtiter plate, letting the antibody bind to the virus
5. An anti-ACE2 receptor IgG, labelled with an enzyme is added to bind with the antibody.
6. The enzyme-substrate is then added. The substrate changes colour when it becomes an enzyme-substrate complex after binding to the enzyme on the anti-ACE2 receptor IgG.

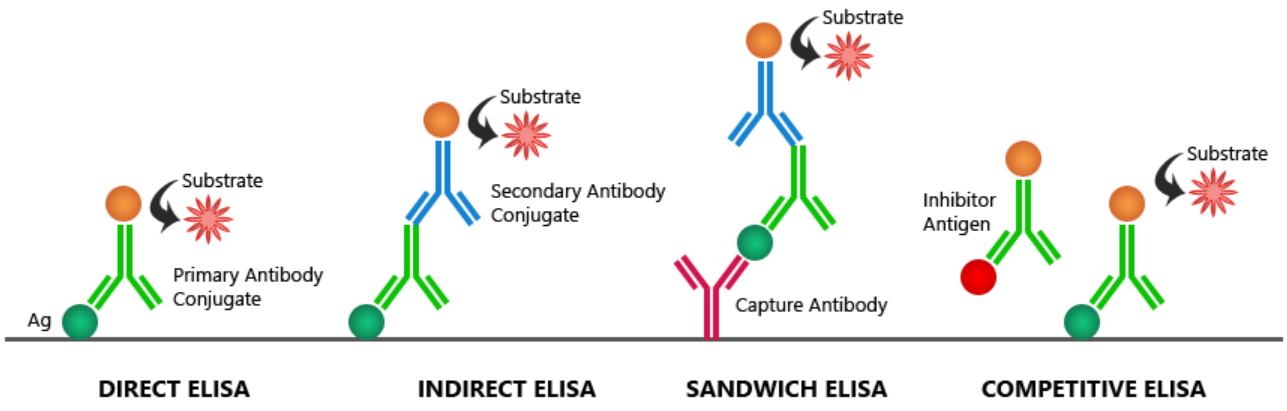


Figure 19 Mechanisms of the 4 types of ELISA

There are 4 major types of ELISA tests; direct, indirect, sandwich, and competitive. They all test for particular molecules by using the matching antibodies and uses Enzyme substrate to indicate the presence of antigen with a change in colour, but their mechanisms are very different[68]. All these types of ELISA have their advantages and disadvantages such as time consumption and accuracy, so different test samples uses different types of ELISA.

[65] Engvall, E (1972-11-22). "Enzyme-linked immunosorbent assay, Elisa". The Journal of Immunology.

[66] Spence, Zachary (2018-10-18). "Biochemistry 8th ed - Jeremy M. Berg"

[67] Serology-based tests for COVID-19, Johns Hopkins Bloomberg school of public health, accessed on 23/04/20, https://www.centerforhealthsecurity.org/resources/COVID-19/serology/Serology-based-tests-for-COVID-19.html

[68] Four Types of ELISA, CUSABIO, published on 2018-07-02, https://www.cusabio.com/c-20659.html

Figure 19- Which ELISA Is For You?, Boster antibody and ELISA experts, 28/07/2017, https://www.bosterbio.com/newsletter-archive/20170728-which-elisa#

Precautions

Washing hands with soap

Using soap to wash hands is one of the simplest ways to kill the virus. Soap molecules have a polar head and a non-polar tail, meaning one side of the molecule is hydrophilic (attracted to water) and the other is hydrophobic (attracted to oil and fats). When soap is added to water, the soap molecules will assemble into bubbles called micelles, pointing the non-polar tails inwards and exposing the polar heads to the water.

SARS-CoV-2 has a lipid bilayer membrane made from phospholipids, which have non-polar tails and do not interact with water molecules. Hence, the virus is not effectively washed off your hands by just using tap water. By adding soap, the hydrophobic tails of the soap molecules interact with the virus membrane. The virus membrane is weakened and slowly pulled apart as the soap molecules bind the virus membrane to the water molecules. The water tension eventually ruptures the virus membrane and kills the virus, at last everything is washed away to the sink[69].

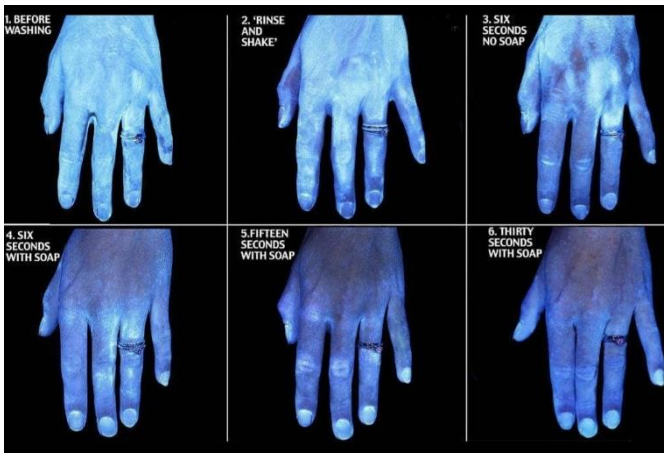


Figure 20 Picture taken under UV light shows amount of dirt on hands after washing hands with soap in different lengths of time

The longer you wash your hands the higher chance of killing the virus, as it requires time for the soap molecules to bind with the virus membrane[70]. It is recommended to wash your hands for at least 20-30 seconds to effectively wash off the virus[71].

Alcohol-based hand sanitizers

Alcohol-based hand sanitizers are effective against killing enveloped virus, such as SARS-CoV-2. The mechanisms of how alcohol-based hand sanitizers kill viruses are similar to how washing hands with soap works; Alcohol molecules bind with the lipid on the virus membrane and cause the membrane structure to denature. This destroys the envelope of the virus and kills it[72].

[69] How Washing Hands with Soap Destroys the Coronavirus, Global handwashing partnership, published on April 8, 2020, https://globalhandwashing.org/how-washing-hands-with-soap-destroys-the-coronavirus/

[70] Fighting Coronavirus with Soap, RCSB Protein DataBank, published on Mar 19, 2020, https://www.youtube.com/watch?v=s2EVlqql_f8

[71] Everything you need to know about washing your hands to protect against coronavirus (COVID-19), UNICEF, published on 13 March 2020, https://www.unicef.org/coronavirus/everything-you-need-know-about-washing-your-hands-protect-against-coronavirus-covid-19

Figure 20- CORONAVIRUS: BLACK LIGHT PHOTOS SHOW IMPORTANCE OF WASHING HANDS AMID OUTBREAK, Sarah Young, Wednesday 18 March 2020

[72] Coronavirus: How hand sanitisers protect against infections, Author: Compound Interest, Explorations of everyday chemical compounds, published on March 4, 2020, https://www.compoundchem.com/2020/03/04/hand-sanitisers/

Alcohol-based sanitizers contain 60-95% alcohol. Most contain either ethanol, n-propanol, isopropanol, or a combination of these[73]. Hand sanitizers with under 60% alcohol are not effective, and above 95% is not effective as water is required to kill the virus on the skin. Handwashing with soap and water is still generally more effective of killing viruses than Alcohol-based sanitizers[74].

Masks

Masks act as a physical barrier, filtering harmful particulates, bacteria, and viral droplets. Studies have shown that wearing face masks is effective against viral transmission by preventing droplets from entering the respiratory system and preventing exhalation of viral droplets to the air. Wearing a surgical mask reduces influenza virus release to the air to a third[75]. This suggests surgical masks could also be effective against SARS-CoV-2 transmission, as both viruses transmit through droplets through the air.

A typical surgical mask contains 3 layers[76]. Spunbond Polypropylene (SBPP) water repellent outer layer prevents water droplets from entering the face mask / entering the middle layer. The middle layer is the filter layer made of Melt-blown Polypropylene (MBPP). It is the most important layer as it filters off droplets and particulates from 0.1 - 100 μm, and it is also electrostatically charged, causing particulates to stick to the filter fibre using static energy. The inner layer is also made of SBPP, but instead of repelling, it absorbs the moisture from breath, which helps prevent the downgrade of the efficiency of the MBPP.

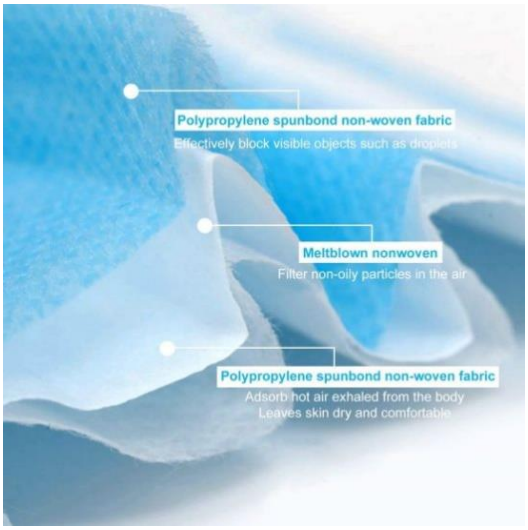


Figure 21 Cross section of a mask, labeling the 3 layers

ASTM standards

American Society of Testing and Materials (ASTM) is an international standards organization that develops and publishes voluntary consensus technical standards for a wide range of materials including surgical masks. These standards must be met to preserve the quality and efficiency of the surgical mask. The following are the main standards that reflect on the efficiency of viral filtration:

[73] Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory Committee, Guideline for Hand Hygiene in Health-Care Settings, October 2002

[74] "Show Me the Science – When & How to Use Hand Sanitizer in Community Settings | Handwashing | CDC". cdc.gov. 3 March 2020. https://www.cdc.gov/handwashing/show-me-the-science-hand-sanitizer.html

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BFE: Bacterial Filtration Efficiency (BFE) measures how well the mask filters out bacteria when challenged with a bacteria-containing aerosol. According to ASTM, a minimum 95% bacteria filtration rate on aureus size 0.6-0.8 microns is required.

PFE: Particulate Filtration Efficiency (PFE) measures how well the mask filters sub-micron particles with the expectation that viruses will be filtered similarly. The higher the percentage, the better the mask filtration. A minimum of 95% particulate filtration rate on the particle size of 0.1 microns is required.

Fluid Resistance: Fluid resistance is the mask ability to minimize the fluid spread from the outer layer to the inner layer. ASTM recommends a minimum of 80 mm Hg fluid resistance against synthetic blood.

Delta P: Pressure Differential (Delta P) is the measure of the breathability of the surgical mask. Delta P is measured in units of mm H2O/cm2, and the ASTM standard of Delta P of less than 5.0

Copper filtered Mask

Copper has antimicrobial properties[77], that's why some masks nowadays have an extra thin layered copper filter beside the MBPP. Copper kills bacteria and viruses by breaking the S-S bond in cysteine, causing their peroxidation and opening holes in the virus membrane, therefore killing viruses. Also, copper complexes can form radicals[78] that alter the three-dimensional structure of the virus protein, causing the virus to lose its ability to infect; some researchers found out that the SARS-CoV-2 loses the ability to infect after having contact with copper surfaces for 4 hours, which is quite a short period compared to plastic, with a 72 hours lifespan. Although copper has antimicrobial properties, the effectiveness of a mask depends on how much copper is in it; there isn't a significant difference between copper filtered mask and normal surgical mask's filtration efficiency[79].

In Hong Kong, wearing masks in public in the COVID-19 epidemic is widely accepted, as wearing masks in public has been ingrained in Hong Kong people since the SARS-CoV epidemic of 2003, and this habit of wearing a mask since the first explosion of COVID-19 epidemic has proven extremely effective against virus transmission. By 8th of May 2020, Hong Kong only has 1,045 confirmed cases, and only 4 deaths with a population over 7.5 million people[80], compared to New York, 327,000 confirmed cases and 20,828 deaths with a population over 8.5 million[81]. Although there are lots of factors linking to viral transmission, we can see that the habit of wearing a mask is crucial for reducing the spread of the virus.

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Possible treatment

Currently, researchers are battling against time to develop a cure against the SARS-CoV-2 virus. Until today, there is no specific treatment for COVID-19, but lots of researchers are focusing on vaccines and antibodies. According to the resource from Milken Institute, up to 238 treatments in consideration and 161 vaccines are developing through a clinical trial in 10/06/2020. Out of 404 possible treatments, 64 of them (15.8%) are looking into developing antibodies.

Convalescent plasma is one of the possible treatments for COVID-19. Convalescent plasma is the blood plasma from COVID-19 recovered patients[82], this contains antibodies to SARS-CoV-2 and can be transfused to infected patients to speed up recovery. Convalescent plasma treatment has been used for more than 100 years to treat a variety of illnesses from measles to polio, chickenpox, and SARS.

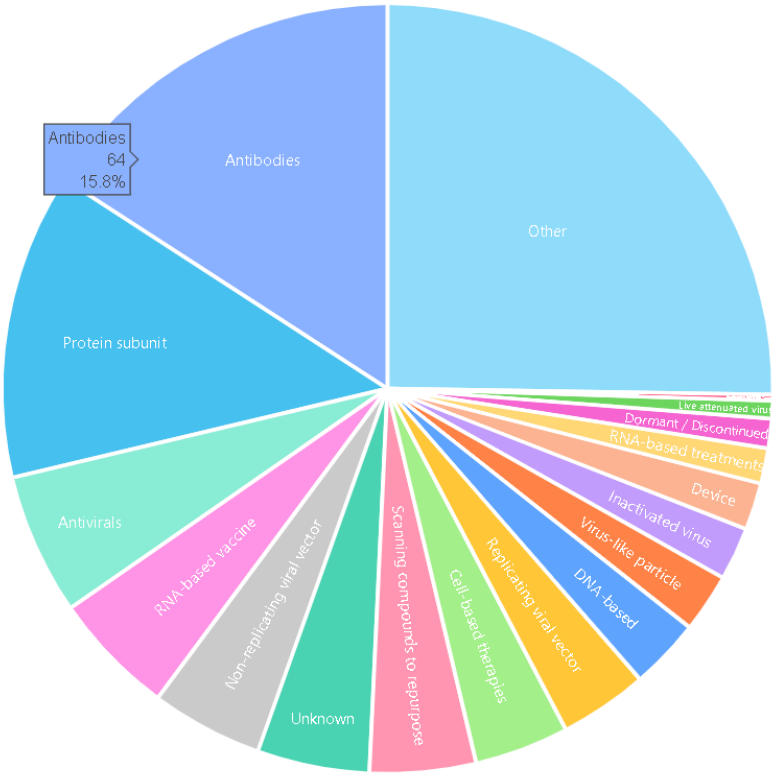

Figure 22 Different types of possible treatments in development

Remdesivir

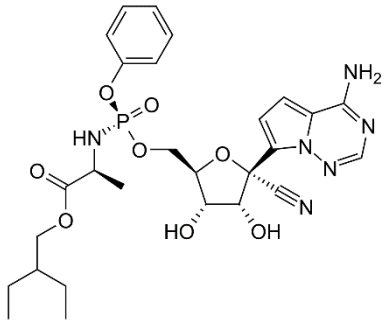

Figure 23 Chemical structure of Remdesivir

Remdesivir is an antiviral medication, originally developed as a treatment for Hepatitis C[83], but it was repurposed to treat the Ebola virus. In April 2020, a study showed Remdesivir reduced the time to recovery for people with advanced COVID-19[84]. Remdesivir is a prodrug; prodrug is a drug that metabolizes inside your body and turns to an active drug. In this case, Remdesivir metabolizes into its active form GS-441524. GS-441524 interferes with the action of viral RNA-dependent RNA polymerase and prevents RNA proofreading, causing a decrease in viral RNA production[85]. RNA-dependent RNA polymerase catalyses the

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Figure 23- The chemical structure of the antiviral drug Remdesivir, Hbf878, 15 March 2020

replication of RNA from the RNA template, and it's crucial at the RNA transcription phase. When viral RNA is not successfully transcribed, the virus can't be replicated[86].

Based on data collected from 1,063 patients, the NIH said patients who received Remdesivir had a 31 per cent faster time to recovery than those who received a placebo[87]. The median time to recovery was 11 days for patients treated with Remdesivir compared with 15 days for those who received a placebo. Recently in April 2020, Remdesivir was viewed as the most likely promising treatment for COVID-19 by Johns Hopkins University[88]. The drug is still currently undergoing trials and waiting for government approval, but statistically, it shows a lot of evidence that it is effective against SARS-CoV-2.

EIDD-2801

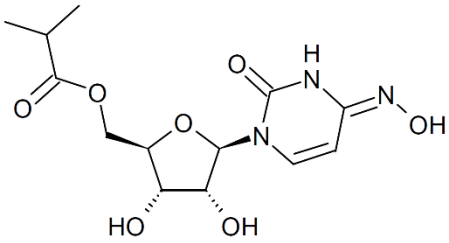

Figure 24 Chemical structure of EIDD-2801

EIDD-2801 is an experimental antiviral first developed to treat influenza[89]. Now it is a potential weapon against SARS-CoV-2, as EIDD-2801 is a non-specific antiviral, which targets lots of different kinds of viruses. EIDD-2801 works by mimicking ribonucleotides – the primary components of RNA molecules. Thus, it sneaks into the process of viral RNA transcription, incorporating molecules in the genome of RNA viruses by attacking RNA-dependent RNA polymerase, which then triggers a cascade of mutations known as viral error catastrophe, which ultimately kill the virus[90].

The mechanism between EIDD-2801 and Remdesivir seems similar, but it is not. Remdesivir works by inhibiting viral replication, but EIDD-2801 works by causing replication errors. However, the main difference between the drugs is Remdesivir must be injected, but EIDD-2801 can be taken orally as a pill. Making it safer and more effective than remdesivir, as people could take it at home rather than in a hospital, which would allow EIDD-2801 to be taken earlier in the course of the disease, killing off the virus before it wreaks havoc on the body.

EIDD-2801 has been shown to treat MERS and SARS (two severe coronavirus infections) in animals when given after an infection has been established. In April, the FDA and the UK Medicines and Healthcare Products Regulatory Agency began Phase I human testing with 100 volunteers, currently still waiting for government approval.

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Figure 24- Structure of EIDD-2801, Meodipt, 28 April 2020

Vitamin C

Figure 25 Vitamin C supplements

Everyday supplements that boost the immune system often get overlooked. Though, vitamins are crucial for our immune system, as they play a pivotal role in maintaining the immune system balance[91].

Vitamin C, also known as ascorbic acid, is not a micronutrient we haven't heard of. Vitamin C has pleiotropic functions related to its ability to donate electrons, meaning it influences immune defence in different ways that do not correlate with each other[92]:

- Supporting various cellular functions of both the innate and adaptive immune system.
- Accumulates phagocytic cells, enhances chemotaxis, phagocytosis, and ultimately microbial killing.
- Needed for apoptosis and clearance of the spent neutrophils from sites of infection by macrophages
- Enhance differentiation and proliferation of B- and T-cells

Vitamin C on Leukocyte function

Vitamin C is a strong antioxidant (reducing agent) in the human body. It can donate an electron to various free radicals in the body, therefore protecting body cells such as White blood cells against oxidative stress from radical reactions[93].

In phagocytosis, neutrophils need to undergo respiratory burst to kill engulfed pathogens. Respiratory burst is the rapid release of reactive oxygen species (ROS), where the leukocyte inflicts oxidative damage towards the engulfed pathogen inside the cell. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is an enzyme that assembles in the phagosome membrane to generate ROS to kill pathogens, ROS is then converted to hydrogen peroxide, which can then be utilized to form the oxidant hypochlorous acid with another enzyme[94]. Hypochlorous acid can also react with amines to form chloramine, and all these oxidants formed from ROS have different reactivity and properties which can inflect oxidative damage towards the pathogen, ultimately breaking down its protein structure.

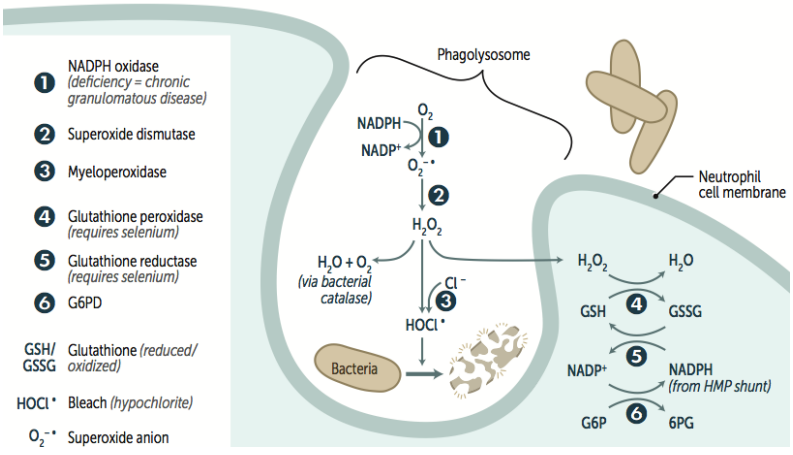

Figure 26 Mechanisms of respiratory burst

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Neutrophils contain high levels of polyunsaturated fatty acids in their plasma membranes, and oxidation of membranes can ultimately cause cell death. For the leukocyte to survive the oxidative stress within the cell, the high concentration of vitamin C is required to sustain the cell's survival by acting as an antioxidant. Vitamin C protein transporters transporter sodium-ascorbate cotransporters (SVCT2) is found on Leukocytes, such as neutrophils and monocytes, actively accumulate vitamin C against the concentration gradient, resulting in fifty to a hundred times higher than vitamin C concentration in the plasma, which is known to protect the cell against oxidative damage[95]. Doing so converts vitamin C to an oxidized state - either as semi-dehydroascorbic acid or dehydroascorbic acid. These compounds can be restored to a reduced state by glutathione and NADPH-dependent enzymatic mechanisms[96].

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex involved in cellular responses to stimuli such as oxidative stress[97]. Oxidants can activate NF-κB, which triggers a signalling cascade leading to the continued synthesis of oxidative species and other inflammatory mediators[98]. Vitamin C has been shown to reduce both oxidant synthesis and NF-κB activation in dendritic cells. Thus, vitamin C could modulate immune function by controlling cell signalling pathways or directly protecting cell structural components from oxidative stress.

Apoptosis

Apoptosis is a form of programmed cell death that occurs in neutrophils. It prevents excessive tissue damage during inflammation, and caspases are the key factor enzymes of this apoptotic process. Caspases are known to maintain cell homeostasis, and during apoptosis caspases ensure the cellular components are degraded in a controlled manner, carrying out cell death with minimal effect on surrounding tissues[99]. They are very sensitive to oxidants (especially ROS generated by activated neutrophils), as oxidants can change the structure of caspases' binding site, therefore vitamin C acts as an antioxidant and protects caspases from degenerating from ROS.

Statistically, vitamin C has been proven useful against upper respiratory tract infection. Many researches have shown that taking 200 mg or more of vitamin C supplementation daily was effective in improving the duration and severity of the common cold[100]. Seriously sick coronavirus patients in New York state's largest hospital system are being given large doses of vitamin C intravenously. Dr Andrew G Weber, a pulmonologist and critical-care (ICU) specialist, said his intensive-care patients with the coronavirus immediately receive 1,500 milligrams of intravenous vitamin C. "The patients who received vitamin C did significantly better than those who did not get vitamin C."[101] Vitamin C isn't a treatment for SARS-CoV-2, but it is evidential to say it's helpful for the patient's immune system during SARS-CoV-2 treatment, and possibly speeds up recovery.

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Conclusion

In conclusion, in this report we have covered;

- The relative roles of structural proteins in viral assembly and the immune response.
- The brief thermodynamics and kinetics behind the S protein and how this may be a cause for higher virulence compared to other coronaviruses.
- Viral mechanisms in pathogenesis, including; cell-binding replication, and entry & exit.
- An insight into the innate and specific immune responses, including the role of IFNs and ILs.
- Transmission & prevention including the effectiveness of masks.
- Possible treatments including Vitamin C, Remdesivir and EIDD-2801.

Understanding these areas is key to creating frameworks for treatment and prevention of SARS-CoV-2, and the information learned can potentially be applied to pandemics in the future.

This report summarises findings strongly based on current scientific research papers, so some information in this report could be incomplete or incorrect as research is still in progress. Discussion on topics such as possible treatments are highly controversial as hundreds of drugs are currently undergoing trial and there is no sufficient evidence on any drug that is effective for treating SARS-CoV-2.

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