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Review

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Glycosylation in SARS-CoV-2 Variants: A path to Infection and Recovery

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ABSTRACT

Glycan is an essential molecule that controls and drives life in a precise direction. The paucity of research in glycobiology may impede the significance of its role in the pandemic guidelines. The SARS-CoV-2 spike protein is heavily glycosylated, with 22 putative N-glycosylation sites and 17 potential O-glycosylation sites discovered thus far. It is the anchor point to the host cell ACE2 receptor, TMPRSS2, and many other host proteins that can be recognized by their immune system; hence, glycosylation is considered the primary target of vaccine development. Therefore, it is essential to know how this surface glycan plays a role in viral entry, infection, transmission, antigen, antibody responses, and disease progression. Although the vaccines are developed and applied against COVID-19, the proficiency of the immunizations is not accomplished with the current mutant variations. The role of glycosylation in SARS-CoV-2 and its receptor ACE2 with respect to other putative cell glycan receptors and the significance of glycan in host cell immunity in COVID-19 are discussed in this paper. Hence, the molecular signature of the glycan in the coronavirus infection can be incorporated into the mainstream therapeutic process.

Keywords: Glycosylation, Glycobiology, Vaccine development, Immunity.

1. Introduction

The COVID-19 pandemic caused by SARS-CoV-2 is the primary health challenge the world has faced for the past thirty months. Coronaviridae is a zoonotic virus family categorised as an enveloped virus that contains positive ssRNA as a genetic material[1]. The genome size of the virus is around 30kDa and comprises RNA as genetic material that binds to the nucleocapsid proteins (N) and forms a complex called a nucleocapsid. The genome mostly codes for four structural proteins: nuclear (N), membrane (M), envelope (E), and spike glycoprotein (S). It also codes for a few other proteins, such as HE, 3a/b, and 4a/b proteins, that help the main proteins perform their functions[2]. The ssRNA also encodes 16 non-structural proteins which actively participate in the viral replication cycle[3]. The nucleocapsid comprises a lipid membrane embedded with the membrane (M) and envelope proteins (E). The transmembrane spike proteins (S) protruded from the surface, giving the crown-like appearance to coronaviruses[4].

Due to the global health emergency, every element of scientific emergence must be investigated to wipe the pandemic off the face of the earth. We can develop an effective prevention method if we thoroughly understand the virus's molecular characteristics and structural and functional roles. Genomics and proteomics have been thoroughly investigated in terms of infection and variations. However, the post-translational modifications, particularly glycosylation, are not a specific gene product or single factor modification, so they have a wide range of effects and diversity on the glycoprotein in defining the function and thus regulating virulence, pathogenicity, and immunogenicity[5–7]. Glycan is also essential in the biological operations of living organisms, including cell-to-cell communication, signal transduction, lectin binding, immunological functions, and cell-to-another-molecule interactions[8,9]. They play a key role in protein folding. The fundamental knowledge of this modification at each region of the glycoproteins of SARS-CoV-2 is not well recognised due to its intricacy, yet it holds significant therapeutic and vaccine potential[10].

Glycan is a biomolecule found in many proteins and lipids as a functional component and was recently discovered on the surface of small RNAs on the surface of living cells[11]. Glycosylation is present in unicellular to multicellular organisms and plays a critical role in biological functions. The main role of glycan in pathogenesis is to change the receptor decoys, increase microbial affinity and stability, and change the glycosylation profile of immunological molecules, which serves as an immunological marker in many diseases[12,13]. Thus, the glycosylation profile of the host framework contribute to an inevitable role in the severity of the disease. The enveloped virus is one of the most common human pathogens[14]. The cellular assembly uses the host cell membrane to make the envelope. The cryo-EM structure of the SARS-CoV-2 indicates that the spike protein is extensively glycosylated, with a glycosylation pattern comparable to that of the SARS-CoV-1 spike glycoproteins (SGP)[15]. The surface of SARS CoV-2 virions comprises 22–40 irregularly arranged spike glycoproteins (SGP) in a trimeric form that are extensively glycosylated and are a crucial component of the pathogenesis because they bind to ACE2, which adorns the surface of respiratory epithelial cells[16]. In SARS CoV-2, glycans may regulate their functional activities like viral attachment to the ACE receptors, membrane fusion, and cellular entry[17–19]. Numerous viruses and pathogens enter the cell by binding through cell surface glycan[20]. Scientists showed that the terminal sialic acid, high mannose, glycosaminoglycans, terminal galactose, and histo-blood group antigens could function as the primary or co-receptor for some viral entry and infections[21–23].

In many cases, virus glycans will attach to specific glycan-binding proteins on the host cell surface. Some of these glycan-binding proteins, also known as lectins, can be unique to tissues, playing an essential role in tissue tropism, host selection, pathogenicity, and transmission of viruses[24–26]. The conserved sequences in the proteins or three-dimensional structures may decide the glycan specificity for any virus or viral species[27].

Glycoprotein analysis is quite challenging due to their complexity in structure and polar nature. Cryo-electron microscopy and biolayer interferometry data were combined and subjected to extensive EM analysis to reveal how glycosylation influences the functional role of S-proteins[28]. However, the structural characterization of glycan, its composition at each site (microheterogeneity) and the occupancy (microheterogeneity) is characterised by various mass spectrometric strategies. Mass spectrometry is an effective method for analysing glycopeptides, especially when combined with matrix-assisted laser desorption/ionization (MALDI)-MS [29] and electrospray ionisation (ESI)-MS[30]. Advanced Mass Spectrometry analyses of glycosylated proteins uses three different ways to comprehend the glycosylation profile, which includes the occupancy, type, and site of each glycoform's relative proportion of glycans and their variation: cleaving the glycans and analysing the released glycans and deglycosylated protein separately; (2) directly injecting the glycosylated protein sample into the mass spectrometer; or (3) Trypsin, chymotrypsin, and alpha-lytic protease are commonly used endoproteases to produce glycopeptides including single glycosylation sequon or O-glycosite [31]. Without stringent sample preparation methods in place, it is challenging to acquire significant data because of factors including micro- and macroheterogeneity that lead to a reduced abundance of each variant of a glycoprotein[32]. These can be overcome by glycopeptide enrichment and derivatization. Commonly used glycopeptide enrichment strategies are based on lectins, hydrophilic interaction chromatography, hydrophilic polymers, hydrophilic metal-organic frameworks (MOFs), and boronate affinity-based enrichment. Numerous derivation techniques, including esterification amidation, permethylation, tandem mass tagging, and others, can also be used to improve the glycopeptide's poor ionisation efficiency[33]. The glycan compositions at each of the sites on the SARS-CoV-2 S protein were determined by LC-MS analysis of the glycopeptide pools with high-energy collision-induced dissociation (HCD) or electron transfer dissociation (ETD) fragmentation[34,35] along with an advanced data analysis platform.

In the past thirty months of scientific research about SARS-CoV-2 and the progression of the pandemic, we understand that glycan has a significant role in disease progression and immunogenicity[13,36,37].Since glycan is a dynamic component of living cells, we can anticipate a change in the glycosylation pattern of host cells, plasma, or antibodies during infection[38–40]. The reflected glycosylation changes in the specific proteins can also indicate the severity of COVID-19. A detailed analysis of the glycan-binding features of SARS-CoV-2 and ACE2 receptors can be a critical element in developing antiviral strategies. In this study, we try to understand the role of glycosylation in the transmission of SARS-CoV-2 infection and the host immune response. N-glycans are thought to affect spike binding to the host ACE2 receptor by maintaining its open conformation and allowing the host to evade immunity. The importance of both the host and viral N-glycosylation pathways is essential in COVID-19 pathogenesis. The study found that using RNA interference or inhibitors to reduce host N-glycosylation, such as tunicamycin or other glycosyltransferase inhibitors, reduced the intensity of the spread of the infection, including variants B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), and B.1.617.2 (Delta)[41]. The study revealed that the cells produced fewer virions under these conditions, and some of them entirely lost their infectivity. Furthermore, partial enzymatic deglycosylation of intact virions revealed that N-glycans on the surface of the virus are required for cell penetration[41]. This paper looks at how glycosylation affects the proteins of both the host and the virus in COVID-19 infections as a way to prevent and spread the infection.

2. SARS-CoV-2 glycosylation using human cellular apparatus:

SARS-CoV-2 attaches to the receptors on the host cell, such as ACE2, GAGs, various lectins, and TMPRSS2. This makes it easier for the virus to get inside the cell and fuse with the endosomal membrane[42–44]. The incoming genomic RNA is released and uncoated after entry, exposing it to the rapid translation of two major open reading frames, ORF1a and ORF1b[45].

The viral genome gets into the cell and makes two proteins with long chains of amino acids that help synthesise RNA. The incoming genomic RNA is released and uncoated after entry, exposing it to the rapid translation of two major open reading frames, ORF1a and ORF1b. The viral genome gets into the cell and makes two proteins with long chains of amino acids that help synthesise RNA. The new RNAs are built of structural proteins, other auxiliary proteins, and S-protein, which together translate into 26 viral proteins[45]. The individual non-structural proteins (nsps) that make up the viral replication and transcription complex are co-translationally and post-translationally processed from the resultant polyproteins pp1a and pp1ab[45]. Translated structural proteins pass through the ER-to-Golgi intermediate compartment (ERGIC), where they interact with N-encapsulated, newly generated genomic RNA, causing budding into the lumen of secretory vesicular compartments[45]. The glycosylation machinery of the host (mammalian) cells makes the virus glycoproteins undergo N and O glycosylation during transit in the ER, ERGIC, and Golgi. N-glycosylation is a co-translational process that starts at ER and gets further modified at Golgi[46]. Unlike mammalian glycoproteins, viral glycoproteins like S protein have a retrieval signal that causes them to concentrate in the ER, ERGIC, and Golgi where the virus particles are formed and eventually bud[34]. This is the main cause of the high-mannose glycan being generated prominently in viral proteins, which results from the glycosylation process not always being completed by exposure to all the glycosyl transferases in the host system.

Figure 1

3. Features of spike glycoproteins and their role in infection:

Spike protein is a type I transmembrane glycoprotein synthesised in ER, consists of 1273 amino acids as monomers and migrates to the plasma membrane as a homotrimer[47]. The S1 domain binds to the host receptor, followed by the transmembrane domain, which is part of the

viral membrane, and then the C-terminus, which has the S2 domain[47]. At its S1/S2 junction, SARS CoV-2 has a multi-basic PRRAR sequence, making it a unique accessory for invading lung cells, whereas most coronaviruses have a single arginine residue[48,49]. The spike glycoprotein has three hinge points and can rotate and flop around to make it accessible to cell surface receptors. It also easily binds to multiple ACEs[50]. The SGPs (spike glycoproteins) of SARS-CoV-2 have a binding affinity of 2-4 times more than SARS-CoV-1 due to the RBD variations that make them stabilised for binding with ACE2[51]. The spike trimer has mechanistic structural and topological features similar to HIV envelope glycoprotein and influenza hemagglutinin, including the post and prefusion structure[52,53]. When the RBD on the spike protein binds to the ACE2, the membrane proteins of the host cell start to fuse and let the virus into the cell[54]. RBD is primarily responsible for recognising and binding the aminopeptidase N-terminal domain of ACE2. It was discovered that 17 amino acids in an extended loop of RBD engage in polar interactions with 20 amino acids in ACE2[55]. Similarly The S1 and S2 remain conjoined through non-covalent interaction and keep up the metastable perfusion state[23].Antibiotics containing aminoglycoside molecules, including kanamycin and amikacin, as well as polysaccharides antibiotics named acarbose , demonstrated significant interactions with S glycoprotein RBD, making them efficient treatments against COVID-19 infection[56].The aminoglycoside antibiotics also function as defensin releasers and translational inhibitors to mediate antiviral action, hence their expected binding to crucial proteins for SARS-CoV-2 infection[57].They also act as immune modulators against SARS-CoV-2[56].

Unlike in other types of viruses, the spike protein, after binding, loses its flexibility and becomes susceptible to the host cell membrane-bound proteases. TMPRSS2, a transmembrane serine protease II, expeditiously cleaves the S2 domain[58]. The S1/S2 can also get dissociated during cellular trafficking by host cell Furin-like enzymes[52]. Furin is a lung surface protein abundant in the lung tissue compared to other tissues that speeds up cellular access[59]. There is

a unique furin cleavage site at SGP specified by 'RRAR', the sequence that undergoes the breakage and leads to the disassociation of the S1 receptor from the protein at the binding site[53]. The endocytosed SARS-CoV-2 is further exposed and processed by cathepsin L, a significant protease at its late endosomal/ endolysosomal stage[60]. The SGP-ACE2 complex undergoes a series of conformational changes before refolding to a stable post-fusion state, which logically promotes the viral and cellular membranes to merge by membrane fusion for the virus to enter rapidly inside the cell[43]. The rapid ingress protects it to avoid getting trapped in the host endosome[48]. This logically explains how chloroquine, the malarial drug, did not work for SARS-CoV-2 infection in clinical trials after the successful lab trials[61]. SARS-CoV 2 infection causes cells to fuse with neighbouring immunological scouts and form syncytia, allowing the virus to avoid detection by the immune system[16]. The S2 domain is the C-terminal domain of S protein comprised of heptad repeats-1 (HR-1,912-984aa) and heptad repeat-2 (HR2-1163–1213aa), which are responsible for accelerating the membrane fusion; a transmembrane domain (TM-1213–1237) and an intracellular cytoplasmic domain (CD-1237–1273 aa)[47]. A unique sequence in the C-terminal domain of the S-protein inhibits it from getting delivered into the lumen of the ER and results in secretion from the infected cell.

4. S-protein glycosylation:

The S-protein is produced using host cellular machinery to translate the viral mRNA to protein. SGP is modified by co-translational and post-translational processes in the host endoplasmic reticulum, including extensive glycosylation, signal peptide removal, trimerization, and subunit cleavage[50] (figure 1). The structural and functional importance of the N- and O- glycans were partially established in the SARS-CoV with the help of CRISPR-Cas9 glycoengineered cells with different glycoforms on the SGP and ACE2 and developed corresponding SARS-

CoV-2 pseudovirus[62]. The studies revealed that glycans on the SGP have a minimal role in receptor binding but significantly regulate viral entry[62].

Soon after the synthesis, S-protein gets trimerized and moved to the Golgi apparatus, where the post-translational events progress. Here the glycan moiety goes through specific enzymatic activities to shape the glycan into complex and hybrid glycoforms (figure1). The post-translational O-glycosylation also happens at Golgi[63]. An endoplasmic reticulum retrieval signal (ERRS) at the C-terminus of matured S-protein prevents it from entering exosomes and causes it to accumulate at the ER-Golgi intermediate compartment (ERGIC), where it interacts with the M-protein, participates in assembly, and becomes associated with a portion of the viral envelope[54,64]. The glycan can also mediate many non-covalent interactions like hydrogen bonds, electrostatic interactions, and Vander Waals forces, which have not been significantly exploited yet[65]. The regulation of glycan biosynthesis of SGP by blocking the glycosylation event in oligomannosylation stage using genetic methods or chemical interferences by using kifunensine has been shown to reduce the host cell entry of ACE2-expressed HEK2 cell lines[62,65]. Molecular dynamic simulations of the S1 protein-ACE2 receptor complex indicated that different types of N-glycans have varied steric hindrance and coulombic repulsion effects on the S1 protein-ACE2 receptor interaction[48].

The N-terminal sequence of the S protein has a 13 aa long signal sequence that locates the S protein synthesised in the ER membrane and cleaved by the cellular signal proteases in the lumen[66]. The N-terminal S1 domain comprises 14-685 aa, and C terminal S2 contains 686-1273 amino acids[67]. The N-terminal domain of S1 (NTD) includes a 14-305aa sequence and has 8 potential N-glycosylation sites (PNGS)with heavy glycosylation[67]. *Amaro et al.* conducted a modulation study by mutating N165 and N234, the two N-glycosylation sites outside the RBD, resulting in the collapsed RBD structure[19]. Stimulation studies clearly show that both sites are required to stabilize the RBD ‘up’ structure. The bilayer interferometry studies further

confirmed that the N165A and N234A mutations have noticeably decreased the RBD binding to ACE2 with a higher impact on N234 due to a conformational shift of the RBD to a 'down' state. RBD sites are made up of the amino acids Arg319-Phe541 (R319-F541)[68]. Two significant N-glycans are found in the RBD at N331 and 343; additionally, two O-glycans are found at T323 and S325, which can significantly regulate the RBD's functionality[47,69]. Hence, we anticipate that glycosylation is one of the critical targets to be considered in receptor binding, and variation may also significantly impact immune evasion, antibody binding, and neutralization.

5. SGP glycosylation is a functional target for SARS-CoV-2 infection.

Glycan-mediated viral entry is ubiquitous for various viral pathogenisis. The SARS-CoV-2 glycan is important for the virus to attach to the ACE receptor, for membrane fusion and cellular access, for replication, exocytosis, immune recognition, innate and adaptive immune response, vaccine design, drug discovery, and therapy[34]. There are around 332 high-assured protein-protein interactions between the virus and human proteins that have been discovered during cellular entry, trafficking, transcription, translation, and regulation of ubiquitination in SARS-CoV-2 infection[70]. In many interactions, glycan, as a part of surface proteins, plays a significant role. Glycan-binding protein domains, such as galectin-like domains, were discovered on the NTD of many coronaviruses' S1 domain, which enhances the virus's tropism via specific glycans on the host cell surface, such as N-Acetyl-9-O-acetylneuraminic acid[71]. During the first stage of the co-translational events at RER, the protein gets embellished with the oligosaccharide in the high mannose form[72]. The regulation of glycan biosynthesis of SGP by blocking the glycosylation event oligo mannosylation stage using genetic methods or chemical interferences by using kifusenine has been shown to reduce the host cell entry of ACE2[62]. Most of the SGP glycans are categorised into the complex glycans being core

fucosylated, and a large portion is neutral with N-acetylglucosamine (GlcNAc) or N-acetylglucosamine. However, these unique epitopes have specificity for different lectin molecules in the host system. The existence of LDN and its fucosylated LDNF derivative and LN, 3′SLN, and 6′SLN terminal moieties on SGP were identified in NMR-based studies, proving that it acts as an epitope for human lectins[37,73]. The terminal side of N-glycans with higher N-acetyl-glycosaminylation and hyper-fucosylation at the RBD reveals glycan-epitopes not seen in MS-based studies[69,74]. High mannose structure was found in abundance at both sites with less complex structures, according to MS analyses[69,73].

14 out of 22 potential glycosylation sites are predominantly occupied and processed to form complex glycans, and the other eight were occupied with oligomannose[34]. The glycosylation sites N165 and N234 were proximal to RBD[19]. However, deglycosylated RBD biolayer interferometry showed a nearly similar affinity to S-protein binding, suggesting that glycosylation does not affect the RBD binding with ACE2. The perfusion structure of the SARS-CoV-2 ectodomain related to the HIV envelop ectodomain showed relatively more minor N-glycan on the surface than HIV[34,53]. The glycan can be a potential immunogenic epitope on SGP and may be able to induce humoral immunity[12,37].

However, the glycan amino acid interaction and glycan-glycan interaction with the ACE-receptor are crucial for a better understanding of the epitopes. Furthermore, since the spike protein is a glycoprotein, it is critical to address the variation and effect of glycosylation in proteins. The majority of the nAb have RBD as their primary binding site[75]. The spike protein additionally contains many O-glycosylation sites, where glycan gets added to the side chain oxygen molecule of Ser or Thr[76]. Typically, the glycans protrude outwards from the SGP trimeric configuration[51] S-protein has a molecular weight of 180-200kDa structurally consisting of an N-terminal extracellular domain, embedded transmembrane domain and an

intracellular C-terminal domain. S protein generally exists in a prefusion metastable form on the virus[53]. The majority of the 22 N-glycosylation sites are included in the conserved region. Thirteen glycosylation sites reside in the S1domain[68,77,78]. The remaining nine sites are in the S2 domain, which completely overlaps with the SARS-CoV-1 sequences[74,76]The S1 domain glycosylation sites share sequons with SARS-CoV-1[74]. *Watanabe et al*. discovered that most of the time, all 22 PNGS are filled, primarily by high-mannose or complex N-glycans, with a low abundance of hybrid glycans[34]. On the other hand, Shajahan et al., discovered partial N-glycan occupancy on 17 of 22 N-glycosylation sites solely with high-mannose and complex-type glycans, with no hybrid type N-glycans[69]. The oligomannose type O-glycans are commonly found at N234, which is at RBD and N 703, which is very close to the S1/S2 joining. The N-glycosylation site at N61, N122, N331, N343, N603, N616 and N717 have a mixture of complex and high mannose glycan types and the remaining N17, N74, N149, N165, N282, N657, N709, N801, N1074, N1098, N1134, N1158, N1173and N1194 has complex glycan type[34,69]. The complex glycans are composed of core fucosylated, terminal galactosylated, non-galactosylated and terminal sialylated structures. Based on the glycosylation site and kind of glycosylation, it can numerously influence the function, including the immunogenicity, vector binding, nAb binding, etc[79]. The biolayer interferometry and cryo-EM analysis prove that the N343 glycan, together with D405, R408 and D427, participate in a gating role in the facilitation of RBD opening[70]. Only a few O-glycosylation sites were discovered and mainly reported at T323 and S325 in the S1 domains of the S-protein, which may participate in antibody recognition, protease priming from the host system and S protein structural integrity and folding[19,69,80,81]. Advanced mass spectrometric methods likewise recognized a unique O-glycosylation site at T678 and additional 8 O-glycopeptides by the studies of Sandra et al [76]. A recent study discovered 17 O-glycosylation on S-protein in a cluster (figure 2). The S1 domain has 11 of these, while the N-terminal of the S2 protein had the remaining six[82]. The findings

show that mutations in N616 eliminated O-glycosylation on T618, implying that glycosylated Asn is required for O-glycosylation linked with N-sequon[82]. They also proposed an "O-Follow-N" rule, in which O-glycosylation occurs near the glycosylated Asn in the N-sequon, which is true in the case of spike protein, as the study reveals that S60, T124, S151, T236, T604/S605, T618, S659, T1076, T1077, S1097, and T1100 are among the 17 discovered O-glycosites that are situated at the consensus sequence or close aminoacid of N-glycosylation[82].

6. M, N, E glycoproteins

In SARS-CoV 2, M, E, and N proteins are also glycosylated, in addition to the S protein. The M protein has 222 amino acids and three membrane-spanning domains at its N-terminus. It is the most abundant envelope protein in SARS CoV-2 and is required for viral particle assembly[83]. The coronavirus budding mechanism is dependent on the M protein. The M protein interacts with the N, E, and S proteins during viral particle assembly[84]. The M protein is glycosylated in all coronaviruses, either by N-linked or O-linked oligosaccharides[84]. The nucleocapsid (N/NCP) protein of SARS-CoV-2 is an RNA-binding protein essential for viral genome packaging on NCP; high-resolution mass spectrometry analyses revealed two N-glycosylations. NTD is glycosylated at N77, and CTD is glycosylated at N269 on NCP, but there is no evidence of N-glycosylation at the other three locations (N47, N192, and N196)[85]. The E protein aids in virus packaging and replication, and its absence reduces or eliminates the pathogenicity[86].

The E protein is involved in various viral processes, including membrane construction and activation, budding and release, apoptosis, inflammation, and autophagy[86]. The E protein is translated into the endoplasmic reticulum (ER) and accumulates in the Golgi. The E protein has two glycosylation sites, N48 and N66, essential for interaction with other membrane proteins[86]. The E protein monomer then self-assembles into an oligomer that operates as an ion

channel. This protein helps in cellular and viral protein folding and trafficking[86]. The exact functions of glycosylation in these proteins are still unknown.

7. Variants of SARS CoV-2

The S-protein from SARS -CoV-1 and SARS-CoV-2 have around 76% structural similarity. They have slightly different sequences in the RBD, making it bind 2-4 times stronger than the SARS-CoV 1 because this variation stabilizes the hotspots of the hACE2-RBD binding interface[87]. The variations have high global transmissibility. B1 with the mutation 'D614G,' which was found early in the pandemic from China and exhibits increased infectivity with higher viral load in patients and a comparable nAb impact with wildtype, was a pivotal primordial variant of concern (VOCs)[88]. The D614G alteration in the spike (S-D614G) of the first major SARS-CoV-2 variation has been linked to altered conformation, improved ACE2 binding, and higher infectivity and transmission. The effect of these mutations on glycosylation has been the subject of research using advanced mass spectrometry. The N-glycosylation of the wild-type (S-614D) and the variant with S-614G SARS-CoV-2 spike glycoproteins expressed under identical conditions were examined and compared using advanced mass spectrometry techniques. The sequons at N17, N61, N74, N331, N343, N657, N1074, N1158, and N1173 were similar in glycosylation pattern. However, the glycosylation pattern of the remaining N-glycosylation sequons altered their glycan distribution. Sequons at N122, N234, N603, N709, and N801 of S-614G showed reduced complex and hybrid structures[35]. Whereas sequons at N165, N282, N616 N1098 and N1134 expressed higher hybrid and lower complex glycans in the S-614G variant. Sequon N717 did not express any complex sugars for the S-614 G or S-614 D variants, respectively[35]. This study also found that the S-614G mutation decreased the amount of complex-type glycans by up to 45% and increased the amount of oligomannose glycans by up to 33%. Because of the changes to the

amino acids, the N-glycosylation profile as a whole became less complicated.Three sequons in the stalk had identical glycosylation between the S-614G and S-614D proteins, while all the altered-pattern glycosylation sites were observed at the spike head[35]. The study shows that the mutation can change the spike protein's overall pattern of glycosylation. The study shows that the mutation can change the spike protein's overall pattern of glycosylation.

Five variants Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) have been identified as variants of concern so far. All five of the identified VOCs have mutations in the RBD and the NTD, except for the Delta version, all of which share the N501Y mutation on the RBD, which increases the spike protein's affinity for ACE 2 receptors, facilitating viral attachment and subsequent entrance into host cells. The alpha variant, B.1.1.7, is reported to have ten variations in the SGP. The key mutations include Δ69-70, Δ–144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H[85]. The 'N501Y' mutation increases the transmissibility; Del 69-70 and Del 144 enable more successful infection P681H impacts the viral infectivity[86]. The modified sequence makes them more favourable 'up' conformation and helps to increase the viral tropism[85]. The SARS-CoV-2 B.1.1.7 UK strain has evolved increased innate immune evasion.[87]-_Transmission of B.1.1.7 may boost in vivo replication and infection duration. The most prominent domain of the S protein, known as the receptor-binding domain (RBD), comprises the amino acids 319-541[83].

The beta variant is developed by mutating the alpha strains further, resulting in significant functional variations. L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G and A701V are important mutations in beta. The three critical mutations, K417N, E484K, and N501Y are on the RBD site. The E484 K mutation causes immune evasion K417 N, which causes enhanced host cell binding[85]. The gamma variant P1 has L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y and T-1027I and V1176F[85]. The key

mutations E484K with antibody escapism, and K417T help the virus for better binding and N501Y with high transmission[88]. The delta variant has the primary critical mutation at T19R, Δ157-158, L452R, T478K, D614G, P681R and D950N[85]. The changes in the sequences can also contribute to the differences in three-dimensional structure and have an increased chance of variant-dependent differential glycosylation, which is yet to be investigated. The interactions of the vital amino acid with adjacent glycan moieties in mutant and wild types also called for the requirement for glycosylation research in SARS-CoV-2[85].

Figure 2:

The Omicron type almost overtook Delta as the most common SARS-CoV-2 variation worldwide[89,90]. Compared to the Delta variant, Omicron has 13 times more infectiousness and 2.8 times more infectiousness. The Omicron (B.1.1.529. BA.2) SARS-CoV-2 mutation, first detected in November 2021, was swiftly identified as a variant of concern that could spread globally[89]. It has 37 mutations, including A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212I, ins215EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F[91]. All the omicron subvariants showed lower antibody neutralization efficacy than the other variants of SARS-CoV2. The neutralisation of BA.1.1 and BA.2 was comparable to that of BA.1 (within a factor of 1.5). BA.2.12.1 had lesser neutralisation by a factor of 1.4 to 1.7 (relative to BA.2, which contains an additional L452Q mutation in its RBD). The neutralising antibody titers against the subvariants BA.4 and BA.5, which are currently prevalent in South Africa and may be the next pandemic subvariants globally, were lower in each of the vaccine groups by a ratio of 2.1 to 2.6 than titers against the subvariant BA.2. This result demonstrated that two mutations (L452R and F486) in the RBD resulted in reduced antibody neutralisation when compared to the RBD

in the BA.2 subvariant[91]. The gamma variant has only 12 mutations on the spike. The mutations N501Y, D614G, K417N, and T478K, are also seen in other variants of concern. There are 15 mutations on the spike area at RBD and eight mutations on NTD, which is primarily responsible for immune evasion and higher binding capacity with ACE2[92]. However, the mutation at the S2 domain is not considered a variant of concern. It is challenging to design a variant-specific vaccination due to the quick emergence of new variants.

Figure 3:

The sequence analysis has revealed that there is no evidence of mutation at the reported or predicted glycosylation sites on the spike proteins. However, the conformational changes due to the amino acid switch may alter the glycosylation enzyme accessibility and hence modifying the glycosylation pattern requires further investigation.

8. Human glycoprotein/glycan receptors or SARS-CoV-2 viral entry

Angiotensin-converting enzyme 2 (ACE2) receptors act as a doorway to the SARS-CoV-1 and SARS-CoV-2 since the spike glycoprotein binds explicitly to the ACE2 on the cell surface for entry. SARS-CoV-2 S glycoprotein interaction with other cell receptors, primarily C-type lectins that identify specific glycan epitopes, host cell glycan receptors and receptor proteases on the host cell surfaces, have been reported in recent investigations, enhancing SARS-CoV-2 entry into vulnerable cells. The main results on SARS-CoV-2 interactions with ACE2 and other cell membrane surface receptors and soluble lectins are critical in viral cell entry, changing infectivity, and perhaps playing a role in future COVID-19 clinical trial manifestations, which are summarised here.

9. Angiotensin-converting enzyme 2 (ACE2)

ACE2 is an enzyme generally found on the epithelial cells of the heart, kidneys, gastrointestinal tract, and blood vessels, in general, creating a protective layer[93,94]. ACE 2 is a zinc-containing carboxypeptidase which is a part of the renin-angiotensin system[95]. Angiotensin is a precursor molecule produced in the liver that gets hydrolyzed by renin at the N-terminal and eliminates ten amino acids to shape ANG I, which is the ligand for ACE I, and gets hydrolyzed by the ACE- I. Two amino acids get taken out from the C-terminal of ANG I to frame ANG II[96]. ANG II expressions generally increase inflammation, pulmonary vascular permeability, and tissue injury damage to the blood vessel lining and blood pressure[97]. ANG II will specifically bind to ACE2 and convert angiotensin II, a multipurpose peptide hormone, to small peptides, angiotensin 1-7, that regulate the functions of cells[93]. However, the RBD of SARS-CoV 2 binds more strongly than the RBD of SARS-CoV1 to the ACE2 receptor[98]. One of the main reasons for this binding is the absence of PNGS at N357 in the SARS-CoV-2 structure[99]. ACE2 and SARS-CoV-2 first infect the respiratory mucosa and then spread to the airways and lungs. The pneumocyte, which is present in the alveoli, and responsible for oxygen and carbon dioxide transfer in the lung alveoli, is highly abundant in ACE2[62]. When the spike protein binds to the ACE2, cells down-regulate the ACE2 expression, upregulating the ACE1 and ANG II expression and causing cytokine and chemokine flux and inflammation[100].h ACE2 is a type I membrane glycoprotein with seven PNGS at N53, N90, N103, N322, N432, N546 and N690[17,18,101]. A recent publication talks about the discovery of an O-glycan at T730[17]. Understanding the degree of macro and microheterogeneity of ACE2 and SGP is essential. The glycosylation site, composition and percentage will elucidate their binding effect for designing the therapeutic strategies and understanding the impact on viral cell passage. The glycan components can also participate actively in bond formation with viral proteins and glycans, apart from the steric effects.

When ACE2 and SGP bind together, the glycan-glycan and glycan protein interactions are challenging to understand, and they may regulate the interaction even when it resides outside of the binding site[17]. Since the N90 and N322 glycosite lie at the S protein binding site of ACE 2, we assume the glycosylation pattern has a significant role in binding[99]. The deglycosylated ACE2 has not shown a considerable effect on SGP-ACE2 binding[102]. N90 has a shielding effect at the RBD SGP interphase, which gets cleared off by its removal; hence, the specific removal of N90 shows the enhancement in the binding with SGP[17]. Glycan -glycan interactions between the two glycoproteins can also possibly alter the binding pattern. The studies have already shown the interaction between the glycans at N546 of ACE to the N74 and N165 of the SGP[18]. In addition to that, glycan of N 90, N 322 and N 546 have also reported interacting with the amino acids of SGP[103]. The detected T730 is at the distal side of the binding site, which may not influence the SGP-ACE binding[101].

10. Heparin

As sialic acid is a key receptor for the human influenza virus, several glycan receptors play an active role in viral pathogenesis[104–106]. Through several in silico and molecular modelling, we can predict that SARS-CoV-2 can bind to charged sugars like sialic acid and sulphated glycosaminoglycans, especially heparin sulphate[15,107–110]. Non coagulate compounds like heparin are used to inhibit SARS-CoV-2 binding and infection[111].

According to Clausen et al., heparan sulphate is an inevitable co-receptor for SARS-CoV-2 infection[110]. Both heparin sulphate and ACE2 bind at the RBD of SGP[109]. It is also proved that the ACE2 and HS binding are co-dependent, and HS binding enhances the ACE 2-binding and supports the viral entry into the cell[15,111] They reveal that heparan sulphate interacts with the receptor-binding domain of the SARS-CoV-2 spike glycoprotein, which is close to ACE2, causing the spike structure to open and allow ACE2 to connect to it[111]. According to Kim et

al., the host cell HS attaches to the SGP first, increasing the accessibility of the host cell surface proteases to digest the S protein, causing the host cell receptor binding to ACE2 to change its conformation, resulting in viral-host cell membranous interaction[112]. Both HS and ACE2 interacts with RBD of SGP. There are mainly four categories of GAGS found in human as Heparin/ Heparin sulphate (Hp/HS), keratin sulphate (KS), and hyaluronic acid and chondroitin sulphate/dermatan sulphate (CS/DS)[113]. HS and CS GAGs are abundant in human lung cells, and Hp is abundant in mast cells. Glycosaminoglycan binding is standard in various coronavirus entry pathways in animal cells due to its proper physiological locations and wide availability in the animal cells [14].The ability of the S glycoprotein RBD to interact with various cell types was inhibited by heparin and HS. Heparin and HS are therefore included in crucial medications to lower SARS-CoV-2 infection[114]. According to recent research, low-molecular-weight heparin usage lowers mortality in individuals with severe coagulopathy caused by the coronavirus[115]. Apart from ACE2, heparin sulphate and dipeptidyl peptidase 4 are the other critical receptor in MERS and SARS infection[41,42.] In SARS-CoV-2, there are three major heparin sulphate binding sites detected. The Major site was at furin cleavage 'PRRARS' from 681-686 amino acid residues. The very common mutation P681H can also impact the HS binding of SGP. The second one is from 453-459, sequences as YRLFRKS. The third site is 810SKPSKRS816, and three different sites of possible attachment increase the possibility of heparin-binding with host cell [113]. Heparin sulphate and sialic acid on the cell surface intricate the viral binding by SGP attachment[43]. The SGP is binding to the heparin through sulphation dependent approach, and it is autonomous of its chain length[10]. Also, the heparin-binding may stabilize the open SGP conformation and subsequently promote the ACE2 binding[44]. Similarly, the mechanism behind thrombocytopenia and thrombosis in coronavirus disease 2019 (COVID-19), particularly among critically ill patients, remains unknown. Heparin-induced thrombocytopenia may be suspected in such seriously ill COVID-19 patients (HIT)[109].

As a result, the GAG binding characteristics of P681H mutations in alpha and omicron variants, as well as P681R mutation in delta variants, may be altered by replacing the hydrophobic P residue with the H /R residue in the Cardin-Weintraub motifs. P681 H mutation was reported to enhance spike protein cleavage [112]. In addition, Omicron possesses an additional mutation at N679K, which is extremely close to the GAG binding site- 2 discussed above.

11. Sialic acids (NeuAc /NANA)

The heavy glycosylation shields many amino acids and is essential for immune evasion. However, sometimes it can bind with various side chains of adjacent amino acids and may change the binding activity. In addition, the charged sugar residues at the binding site can change the microenvironment near the residue, which may enhance or decrease the binding properties. The receptor-binding domain (RBD) of the spike (S) protein on SARS-CoV-2 identifies oligosaccharides containing sialic acid (Sia), with a preference for monosialylated ganglioside, according to a recent study on host cell glycosylation and its function in SARS-CoV-2 binding[107]. The findings show that blocking sialic acid expression on the cell surface affects RBD binding and that pharmacological or genetic disruption of glycolipid biosynthesis can mimic both pseudotyped and accurate SARS-CoV-2 viral entry. These findings show that sialylated glycans, particularly glycolipids, aid SARS-CoV-2 virus entrance[107]

Tyrosine-protein kinase receptor UFO(AXL)is another well-known host glycoprotein that binds particularly with the SARS-CoV-2 N-terminal domain and promotes infection[116]. Downregulation of AXL, but not ACE2, reduced SARS-CoV-2 infection in lung cells significantly, showing its relevance as a co-receptor in SARS-CoV-2 infection[116].

12. **TMPRSS2**

Host cell proteases activate the spike proteins of the Coronavirus. Hoffmann and colleagues discovered that the pandemic SARS-CoV-2 contains a highly cleavable S1/S2 cleavage site that is absent from closely related coronaviruses. Furin mediates cleavage at this location, which is necessary for viral entrance into human lung cells[117]. The primary host protease that allows entering host cells via spike (S) protein priming is transmembrane serine protease 2 (TMPRSS2)[118]. TMPRSS2 and TMPRSS4 have increased SARS-CoV-2 infection in human small intestine enterocytes[119]. TMPRSS2 is also a glycoprotein which causes the cleavage of SGP to S1 and S2 subunits[48]. One of the other significant consideration locations in amino acid variation is 613-705, which covers the whole S1 part of the S1/S2 junction and a small portion of the S2 side, which includes the furin cleavage site and are specifically crucial in viral attachment and cell entry to the host cell. Hence the N-glycosylation at N616 and N657 may have importance in its transmittance and deciding the viral load. Additionally, O-glycosylation at T618, T676, and T678 on SGP[117] may also affect TMPRSS2 activity. However, this has yet to be determined. The therapeutic application of the development of active site inhibitors Of TMPRSS2 against a variety of respiratory viruses, including Influenza, and coronavirus have

13. Role of glycosylation in host immune response and vaccine design

Glycan has an inevitable role in disease progression and immunogenicity in any disease state. Although the spike protein obeys the genetic information from the virus, the host cell regulates the glycosylation part of the spike protein. S protein glycosylation plays a dual role in host cell immune response. It should be logical to think of the RBD of S glycoprotein as a main target in the SARS-CoV-2 vaccine. Studies have proved that the neutralizing antibodies against S-protein, especially against RBD sites, are seen in COVID-19 after 2-3 weeks of infection[75]. Even though S1+S2 ectodomain, S1 domain and RBD can induce antibody production[120]. RBD elicits higher titre and higher affinity antibody production than other sites, making it a potential

vaccine candidate, while S2 is a poor immunogenic domain[121]. Anhui Zhifei Longcom Biopharmaceutical, CanSino, Gamaleya, Janssen, Novavax, Moderna, and Pfizer/BioNTech are the few companies with advanced or approved vaccines that use RBD as an immunogen or a target that contains RBD or RBD including full-length S glycoprotein[122].After SARS-CoV-2 replication, the S glycoprotein is glycosylated by hijacking the host glycosylation processes, resulting in a unique glycosylation pattern for the host cells[7,81]. After SARS-CoV-2 replication, the S glycoprotein is glycosylated by hijacking the host glycosylation processes, resulting in a unique glycosylation pattern for the host cells[7,81]. Hence, it functions to make a veil of "self" on the virus protein backbone to our immune system. Therefore, glycosylation plays a significant role in the immune escapism of the virus[123]. Coronavirus glycans have a pivotal role in vaccine design as they can interfere with immunogenicity [7,80,124]. Ryo Shinnakasu et al. observed glycan engineering of the SARS-CoV-2 receptor-binding domain elicits cross-neutralizing antibodies for SARS-related viruses, resulting in higher proportions of core-RBD–specific germinal centre (GC) on the B cells and antibody responses, indicating significant neutralizing activity for SARS-CoV, SARS-CoV-2, and the bat WIV1-CoV[124]. These findings have implications for the development of vaccinations against SARS-like viruses. The recently developed HIV-1 vaccines are driven by the knowledge of glycosylation on the envelope protein and its effect on immune evasion and immune responses[21,125]. On the other hand, glycan can camouflage the epitopes and may lead to evading the surveillance of the immune system[126,127].

14. Host glycans as a part of the physiological barrier

The glycoproteins known as mucins, which are often found on human epithelial cells and trap microorganisms via their O-glycans (O-GalNAc), perform a crucial physiological role in eliminating pathogens and particles trapped in mucus and hence act as a physical barrier of the immune system. The mucins and their glycosylation play an inevitable role in maintaining the

health of the respiratory tract, affecting the morbidity and mortality of patients with lung disorders[128–130].

15. Host Glycoproteins in the second line of immune defence

Immunoglobulins are one of the most studied glycoproteins which correlate the functions of antibodies with glycosylation during various infection[131]. As components of soluble glycoprotein, IgM and IgA also contribute to a secondary line of infection in addition to IgG.As components of soluble glycoprotein, IgM and IgA also contribute to a secondary line of infection in addition to IgG. The IgM isotype plays a major role in innate immunity against viral infections, while IgG and IgA are also important soluble components of this defence. Certain viruses, including the influenza virus, the lymphocytic choriomeningitis virus, and the vesicular stomatitis virus, are bound, neutralised, and cleared by natural IgM antibodies[132,133].CDC, neutralization and ADCC effector functions of the immune system can be regulated by antibody glycosylation during infection[134]. Immunoglobulin G (IgG) N-297 Fc glycosylation is essential for the antibody to perform its effector functions, including ADCC, CDC and neutralization. The Fc glycan is highly variable, and expression stays as an interface between genetic and environmental factors. The various clinical manifestations of SARS-CoV-2 infection are highlighted by IgG N-glycosylation[134]. Hence, understanding IgG glycosylation has a vital role in acquiring biomarkers, vaccine development, and immunotherapy knowledge in COVID-19. Recent studies in critically ill COVID-19 patients suffering from respiratory syndrome show severe thrombosis, and the COVID-19 specific cytokine storm worsens the immune responses and may also cause the activation of macrophages to form immune complexes bearing immune complexes afucosylated anti-SGP-IgG[135,136]. The anti-SARS-CoV-2-IgG response might get enacted by the platelet thrombosis on vWF[136]. SGPs are a significant site for antibody neutralization. Their variations by altering amino acids (as in mutations) or their subsequent modification (as variation in post-translational modifications)

can change the neutralizing antibody binding on specific sites of SGP. Around 5-20% of nAbs non-neutralizing Abs of S proteins are targeted against the NTD region[67]. NTD-specific antibodies are crucial in host immunity and target particularly vulnerable sites at NTD and efficiently participate in neutralizing effects. P1 lineages identified with NTD mutation have an inefficient binding effect with neutralizing antibody[137]. There are 8 PNGs placed at the NTD of SGP, and they are N17, N61, N74, N122, N149, N165, N234, and N 284 (figure 2). Neutralization antibody generally binds to the specific amino acid residues by a defined linkage. For example, 4A8 is one of the neutralizing antibodies attaching to the SGP through salt bridge formation with K147 and K150 at SGP. Any alteration that can interfere with the binding may hinder the binding properties[138] The studies have proved that the neutralizing antibodies against S-protein, especially against RBD sites, are seen in COVID-19 after 2-3 weeks of infection[75]. Even though S1+S2 ectodomain, S1 domain and RBD can induce antibody production[120]. RBD elicits higher titre and higher affinity antibody production than other sites, making it a potential vaccine candidate, while S2 is a poor immunogenic domain[121]. In many pathological states, Fc glycosylation can act as a biomarker correlating the pathological and physiological condition[134,135,139]. In HIV, envelope proteins exhibit a cluster of high mannose sugar, and the infected patients elicit the nAb against the viral glycan. In infection with the coronavirus disease 2019 (COVID-19), antibody-mediated platelet activation is a driver of thrombosis[136]. The study on antibody-mediated platelet activation proves that sera from critically ill COVID-19 patients can activate platelets by crosslinking their Fc-gamma (ɤ)-receptor (R) IIa[140]. The studies showed a noticeable difference in serological protein glycosylation in severe COVID-19 patients, including an increase in afucosylated and hyper galactosylated Fc glycan on the IgG1 and it was not contributed by the general antibody population and only specific to the antibody response against the surface membrane proteins of enveloped virus or alloantigens on the blood cells[134–136]. The

afucosylated glycan in IgG increases antibody-dependent cellular cytotoxicity (ADCC) by increasing FcγRIIIa binding of the Fc and activates the proinflammatory cytokine discharge by monocytes[135,136,141]. Low levels of IgG sialylation contribute to the ADCC-mediated increase of inflammatory cytokines in severe instances. Reduced sialylation and enhanced galactosylation contribute to COVID-19 pathogenesis by activating the lectin-dependent alternative complement pathway[134]. Hence IgG afucosylation and hyper galactosylation can be an indicator of the severity of the COVID-19 infection.

An association between blood type ABO and SARS-CoV-2 infectivity were also reported [142].Blood group O individuals exhibited greater resistance to SARS-CoV infection. Cellular models have offered a possible answer for blood type modulation of infection, demonstrating that spike protein/Angiotensin-converting enzyme 2 (ACE2) interactions are inhibited by natural human anti-A antibodies. Therefore, people with blood group other than A, like O or B, which make anti-A antibodies, may be less likely to get COVID-19 because anti-A antibodies stop the virus from spreading. SARS-CoV-2 is a virus that causes severe acute respiratory syndrome[143]. Human lectin binding of SGP glycan can have a variety of roles in various functionalities of the host system, from pathogenesis to immunogenicity. A lectin can be present in the blood or on the surface of cellular membranes. Mannose-binding lectin(MBL) is a soluble blood protein that specifically binds to mannose sugar and plays a greater role in antigen clearance. The oligomannose-type glycans that are present on the surface of the SARS-CoV-2 S protein may also be recognised by it[138]. MBL is an essential part of innate immunity. MBL protects against SARS-CoV-2 infection in its early phases. One of the reasons why children have a stronger immunity to COVID-19 may be because MBL is expressed more in children than in adults[144]. Various pathogen recognition receptors (PRRs) are C-type lectins involved in antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), detecting carbohydrate-based pathogen-associated molecular patterns and elaborating the

immune response[145]. The spike glycoproteins of SARS-CoV-2 have been expressed in human HEK293F cells, and the glycan structures of receptor binding were studied using NMR to understand their role in human lectin binding[146]. The NMR studies revealed that the N-glycans at N331 and N343 of RBD have various binding capabilities to macrophage galactose lectin (MGL) and galectin 3,7 and 8, sialic acid-binding immunoglobulin type lectin (Siglec)-10, and non-integrin that grabs dendritic cell-specific intercellular adhesion molecule-3 (DC-SIGN)[147]. It generally binds specifically to the high mannose and small glycans containing fucose as in Lewis's type glycan[148].Gal-3 is a secreted lectin with strong proinflammatory properties that boost interleukin 6 and tumour necrosis factor production, two cytokines that play a crucial part in cytokine storm-induced pneumonia that results in disastrous outcomes in COVID-19 patients.Gal-3 inhibitor therapy appears promising for lowering the SARS-CoV-2 infection's cytokine storm in patients. a few of the Gal-3 inhibitors belapectin, TD139, and GB1107 are presently undergoing clinical trials[149]. According to the findings, both DC-SIGN and L-SIGN bind to S glycoprotein via Complex N-glycan and oligomannose in the case of SARS-CoV-2[150]. According to the same study, the complex sugar with terminal GlcNAC and core fucosylation as well as Lewis $^{A/X}$ epitope had a significant affinity for both lectins[150]. The binding affinity of ACE2, DC-SIGN, and L-SIGN was also determined, and it was discovered that for SARS-CoV-2, ACE2 had the highest binding affinity, followed by DC-SIGN, and L-SIGN had the lowest[150].

MGL, or macrophage membrane C-type lectin, is another, has been linked to viral pathogenesis[106] and has been proposed as a possible receptor for SARS-CoV-2 cell entrance[108]. MGL recognizes the terminal galactose or GlcNAcs residues on the complex N-glycan and Tn antigens on the O-glycans, especially on RBD, Thr323 or Ser325 of S protein[108]. DCs and macrophages in the upper airways and lungs express MGL. Desialylation exposes the terminal galactose, which boosts MGL binding marginally[106].

16. Glycan-lectin interaction-based immune cell activation

Glycan-binding proteins play a major role in pathogenesis as it specifically binds to the carbohydrate receptors and hence plays a major role in pathogenesis. Siglecs are carbohydrate receptors that bind sialylated glycans and are involved in immune cell signalling. They are found on nearly all immune cells, including white blood cells, and play an essential role in immune cell signalling[151]. Another family of galactose-binding proteins found in the majority of epithelial and immunological cells is galectin. There are different galectins found on human cells[9]. Galectin-3 and 9 is a -galactoside-binding protein found on epithelial cells as well as other immune cells such as DCs, macrophages, and Kupfer cells[108] and found to be COVID-19 biomarker that binds to terminal galactose residues on S protein. Many mannose receptors are found on immune cells that recognize high mannose sugars on the SGP and participate in immune responses in COVID-19[150]. SARS-CoV-2 infectivity was increased by another glycoprotein host factor, neuropilin-1, a well-known cell surface growth factor[152]. Furin, a host protease, cleaves the full-length precursor S glycoprotein into two polypeptides known as S1 and S2[48]. S1 has a polybasic 'RRAR' carboxyl-terminal sequence that adheres to a C-end rule (CendR) motif that binds to cell surface neuropilin-1 (NRP1) and NRP2 receptors[152]. Research shows that blocking molecular interactions with small-molecule inhibitors or monoclonal antibodies reduces viral infection in cell culture[152].

The soluble L-SIGN-Fc inhibited viral entrance by 48%[108]. In COVID-19 patients, plasma galectin-9 was positively linked with a wide range of proinflammatory biomarkers (e.g., IL-6, TNF-), whose expression and production by COVID-19 patient immune cells were boosted by galectin-9 treatment in vitro. Galectin-9 was also downregulated in COVID-19 neutrophils[153,154]. It participates in cell-cell and cell-extracellular matrix interactions and activates a variety of cells, including APCs and inflammatory cells, which it recruits to infected areas to modulate biological response[155]. During SARS-CoV-2 infection, circulating

galectin-3 levels rise, and it could be employed as a predictive biomarker for severe COVID-19 in SARS-CoV-2 infected individuals[153]. Galectin-9 is also a possible biomarker due to its high specificity and sensitivity in distinguishing between SARS-CoV-2 infected and healthy individuals. It is produced by a variety of immune and non-immune cells and regulates a variety of biological processes, including chemotaxis, eosinophil activation, DC maturation, and the function of macrophages[154]. The research also identified CD8+ and CD4+ T cells specific to SARS-CoV-2 from COVID-19 recovered patients[156]. Glycans may also weaken the T-cell reaction. For instance, it has been accounted for that some glycans in SGP may meddle with antigen presentation in an HLA complex[157]. Innate immune cells also have toll-like receptors (TLRs), which distinguish between self and foreign molecules. Toll-like receptors are found on macrophages in the nasal cavity, airway epithelial cells, natural killer cells, and a variety of other cells[158,159]. The mannose receptors are commonly expressed on macrophages, monocytes, and dendritic cells. TLR2, TLR4, and MR collaborate in human monocytes, suggesting that receptor synergism between MR and TLR2 and TLR4 may account for the severe inflammation in COVID-19[108]

The transmission of SARS-CoV-2 to susceptible cells via Siglec-1 was more effective than DC-SIGN-mediated transfection and was successfully prevented by anti-Siglec1 monoclonal antibodies[160]. Siglec-1 on DCs facilitated SARS-CoV-2 trans-infection of target cells, whereas Siglec-1 on macrophages resulted in higher cytokine release after viral capture compared to DCs, triggering proinflammatory responses and potentially participating in a cytokine-storm associated with severe COVID-19 infection [160]. SARS-CoV-2 S glycoprotein binding to Siglec-3, Siglec-9, and Siglec-10 is expressed on myeloid immune cells in which Siglec-3 interacts primarily with 2,6 linked sialic acids and is abundantly expressed on monocytes and macrophages. Siglec-9 is highly expressed on monocytes, neutrophils, DCs, NK, and T cells and binds to 2,3 linked sialic acids on fucosylated or sulfated

oligosaccharides[160]. Siglec-10 is highly expressed on monocytes, B cells, and eosinophils and binds to 2,6 linked sialic acids. These findings imply that Siglecs play a role in modulating the function of monocytes, macrophages, neutrophils, eosinophils, and B cells in COVID-19, potentially contributing to immunological suppression[160].

In addition to immunoglobulin, glycan alterations may play a significant role in inflammatory vascular diseases as the cell surface N-glycosylation of endothelial tissues gets modified according to the proinflammatory responses[156]. The critically affected COVID-19 patients showed elevated levels of proinflammatory cytokines such as IL-1, IL-6, IL-12, IFN-α, and TNF-ɤ usually cause lung cell inflammation and might also exhibit altered glycosylation in the immune cells[161]. The infection triggers the host immune response, which causes the residency of immune cells, and causes the flux of chemokines and cytokines at the site of conditions, eventually causing inflammation. Sometimes the inflammation and the immune response also destroy the host cell, leading to the severity of COVID-19[162].

17. Concluding remarks

The SARS-COV-2 infection is a significant medical challenge even after thirty months of active research in controlling the pandemic. The role of glycosylation in virus-host interaction, host-cell attachment, pathogenesis, and activating the immune system have a pivotal role in developing a personalized treatment strategy and vaccine development. The most dynamic glycosylation changes during the pathogenesis as well as on viral proteins are underexplored in the scientific focus on the study of amino acid changes and various mutations. Here we detail the role of glycosylation in SARS-CoV-2 glycoproteins, ACE-II glycoprotein receptors, and host antibody glycosylation in COVID-19.

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Abbreviations

Nucleocapsid proteins (N); membrane (M); envelope (E); spike glycoprotein (S); SARS-CoV-1 spike glycoproteins (SGP); matrix-assisted laser desorption/ionization (MALDI)-MS28; electrospray ionisation (ESI)-MS; metal‐organic frameworks (MOFs); high-energy collision-induced dissociation (HCD); electron transfer dissociation (ETD); ER-to-Golgi intermediate compartment (ERGIC); N-acetylglucosamine (GlcNAc); variant of concern (VOCs); receptor-binding domain (RBD); chondroitin sulphate/dermatan sulphate (CS/DS); endoplasmic reticulum retrieval signal (ERRS);N-terminal domain(NTD); potential N-glycosylation sites (PNGS); N-acetylglucosamine (GlcNAc); Angiotensin-converting enzyme 2 (ACE2); receptor-binding domain (RBD); sialic acid (Sia); transmembrane serine protease 2 (TMPRSS2); O-glycans (O-GalNAc);pathogen recognition receptors (PRRs); antigen-presenting cells (APCs); antibody-dependent cellular cytotoxicity (ADCC); Mannose-binding lectin(MBL); dendritic cells (DCs); dendritic cell-specific intercellular adhesion molecule-3 (DC-SIGN); sialic acid-binding immunoglobulin type lectin (Siglec); macrophage galactose lectin (MGL); C-end rule (CendR); toll-like receptors (TLRs).

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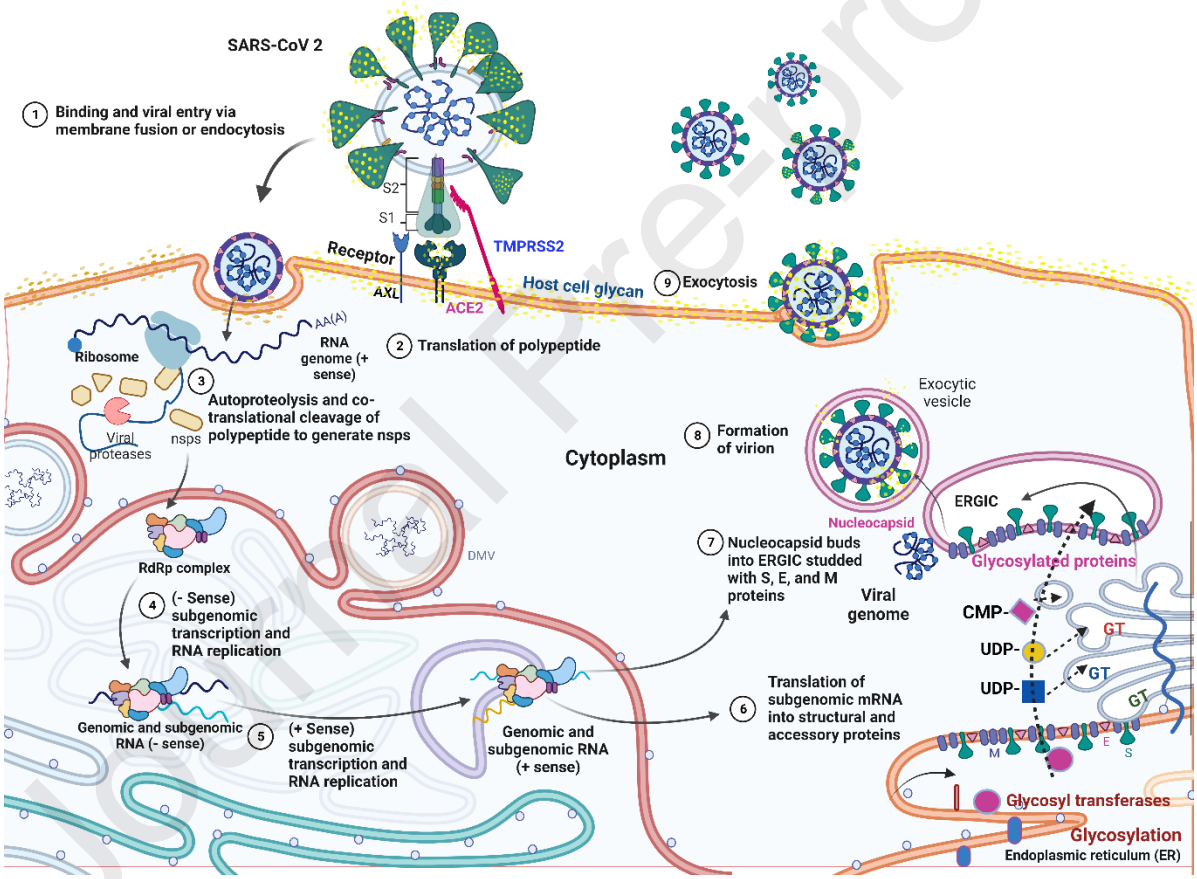


Figure 1:S-protein of SARS-CoV-2 binds to the ACE2 receptor on the host cell and enters via membrane fusion and endocytosis. The viral genome is released into the host cell, followed by viral polypeptide synthesis and autoproteolysis, which results in non-structural proteins (nsps). Nsps control RNA synthesis and translation of subgenomic mRNA to the M, N, Eand *S structural proteins and the accessory proteins. These proteins are subjected to post-translational changes, particularly glycosylation. The glycosyltransferases glycosidases and transporter proteins in the host endoplasmic*

and Golgi membranes supply the nucleotide monosaccharide that acts as a sugar donor to the protein glycosylation. Matured S-protein consists of an Endoplasmic reticulum retrieval signal (ERRS) at the C-terminal domain, which inhibits the protein from exosome entry and makes them accumulate at the ER-Golgi intermediate compartment (ERGIC) The nucleocapsid Buds into ERGIC studded with S, M, and E proteins and assembled to form a virion in an exocytosis vehicle and released from the host cell through exocytosis.Created with BioRender.com]

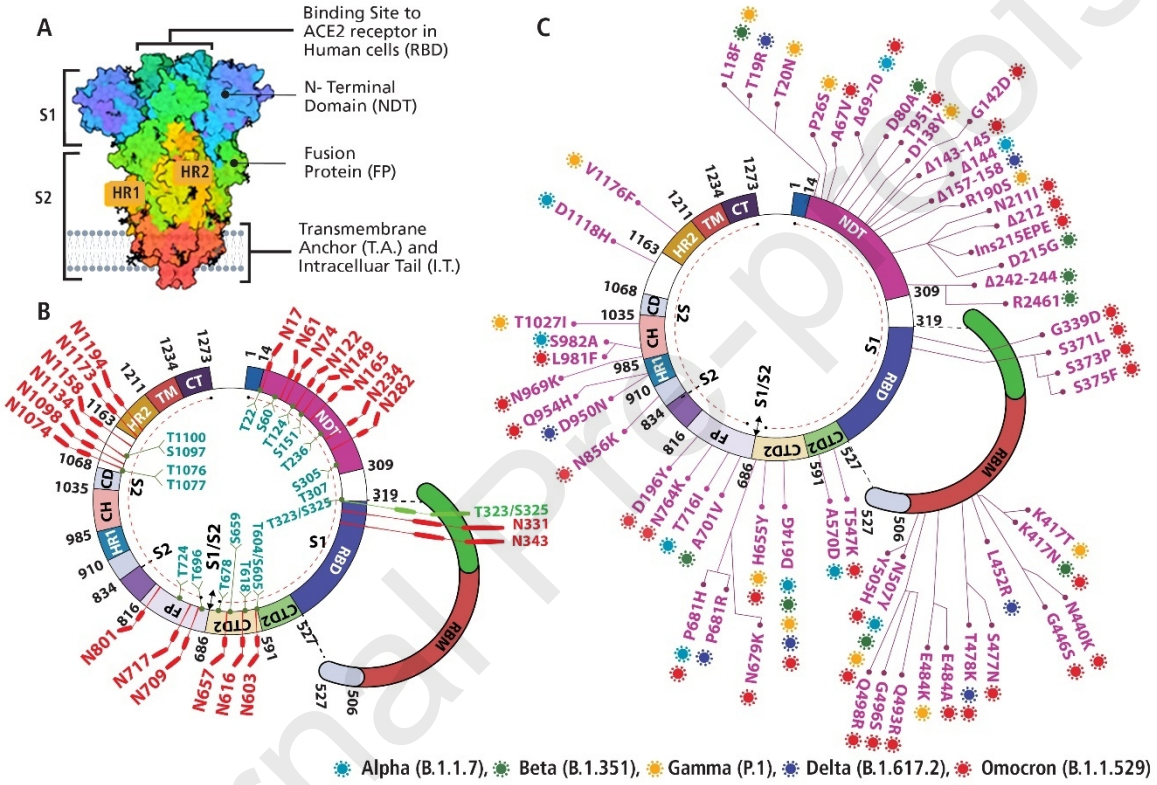


Figure 2A) Major functional domains of SARS-CoV 2 spike protein. B 22 major N-glycosylation sites (labelled in red) and O-glycosylation site (Labelled with Green).c) Mutation regions from the Spike protein amino acid sequences: SARS-CoV-2 and A) Alpha (B.1.1.7), B) Beta (B.1.351), C) Gamma (P.1) and D) Delta (1.617.2) and Omicron (B.1.1.529). NTD: N terminal domain, RBD: receptor binding domain, CTD1 and 2: C terminal domain 1 and 2, FP: Furin protease site, HR1and HR2: heptad repeat site, CH, CD, TM, CT- C-terminal [Created with BioRender.com]

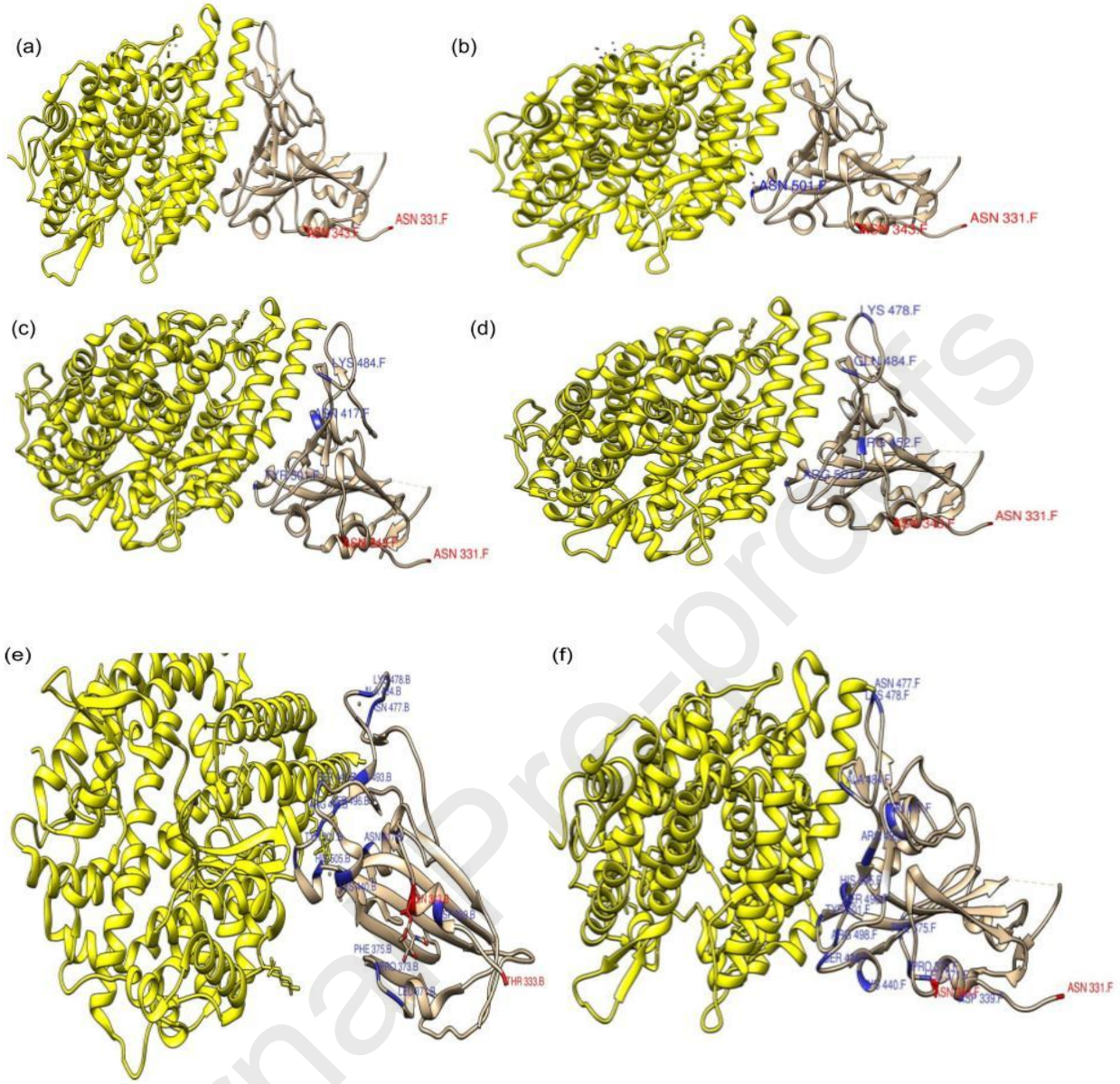


Figure 3: SARS-CoV2 Glycosylation sites (red colour) and Mutations sites (blue colour) of Wuhan strain(A), Alpha(B), Beta(C) Gamma(D), Delta(E) and Omicron(F) variants.

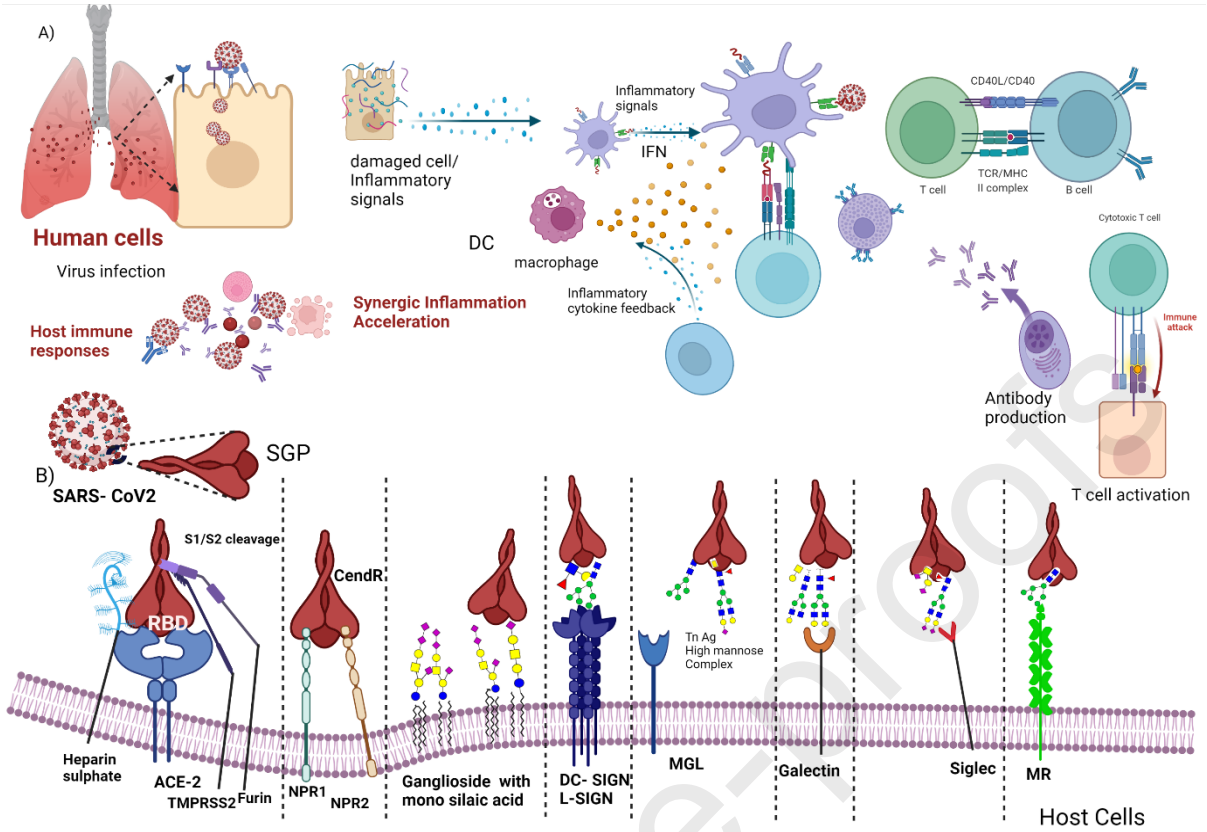
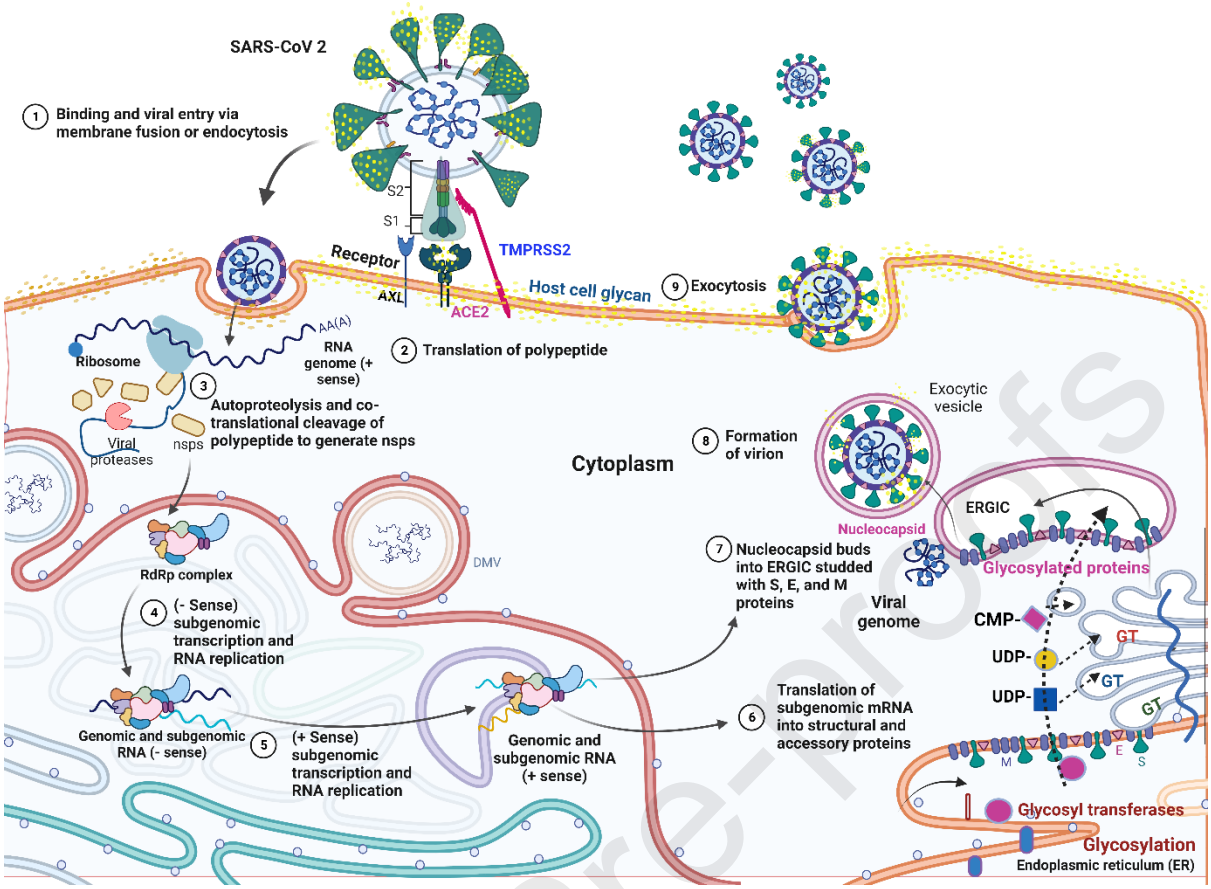


Figure 4: A) SARS CoV2 viral interactions with various glycoproteins in host cells B) critical glycoprotein mediated virus- host cell interactions in SARS-CoV-2 infections and recovery [Created with BioRender.com]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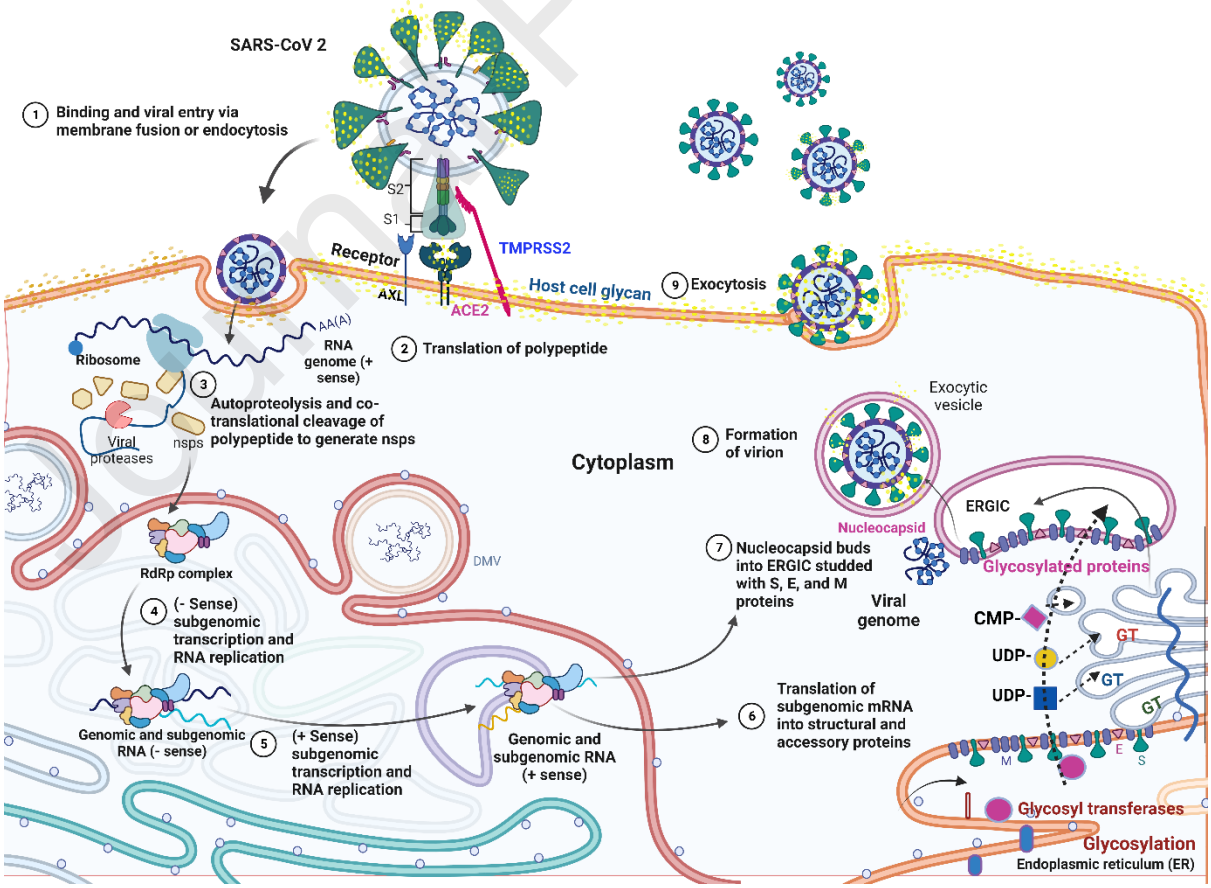
1 Binding and viral entry via membrane fusion or endocytosis
S2
S1
Receptor
AXL
TMPRSS2
ACE2
Host cell glycan
9 Exocytosis
Ribosome
AA(A)
RNA genome (+ sense)
2 Translation of polypeptide
3 Autoproteolysis and co-translational cleavage of polypeptide to generate nsps
Viral proteases
nsps
Cytoplasm
8 Formation of virion
Exocytic vesicle
Nucleocapsid
ERGIC
Glycosylated proteins
7 Nucleocapsid buds into ERGIC studded with S, E, and M proteins
Viral genome
RdRp complex
DMV
4 (- Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (- sense)
5 (+ Sense) subgenomic transcription and RNA replication
Genomic and subgenomic RNA (+ sense)
6 Translation of subgenomic mRNA into structural and accessory proteins
CMP-
UDP-
UDP-
GT
GT
GT
M
E
S
Glycosyl transferases
Glycosylation
Endoplasmic reticulum (ER)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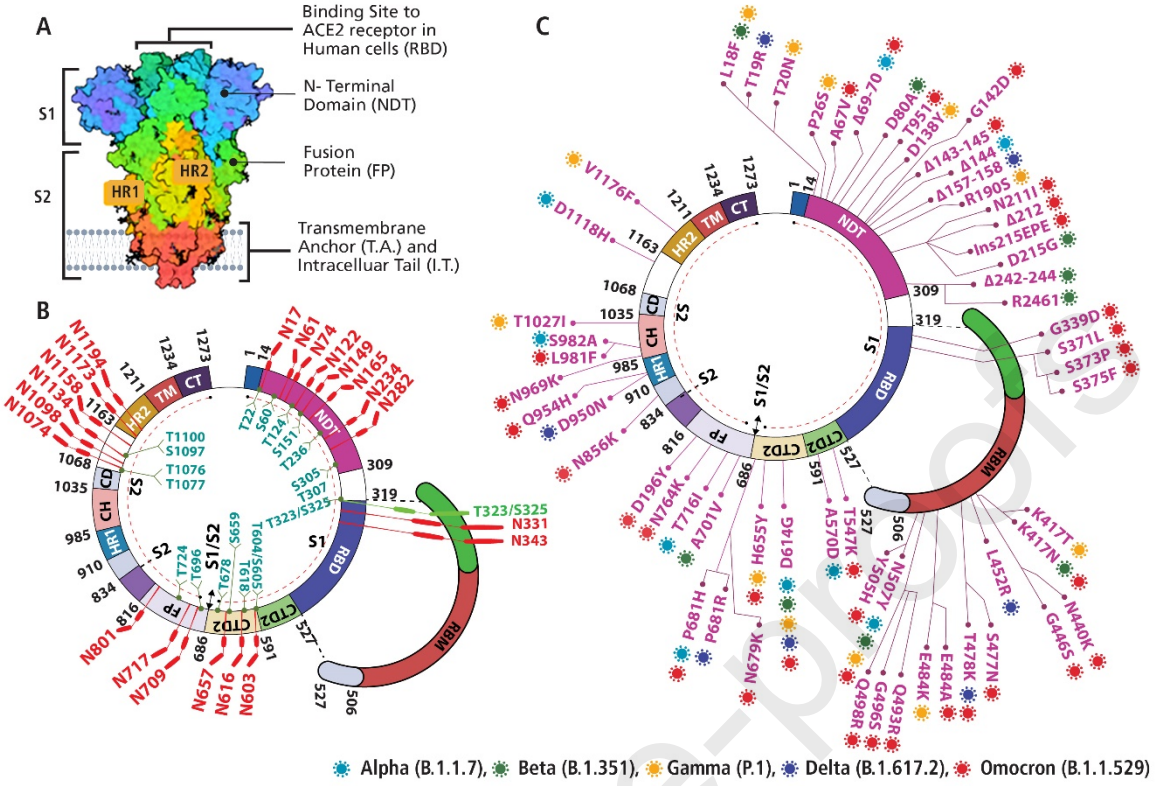

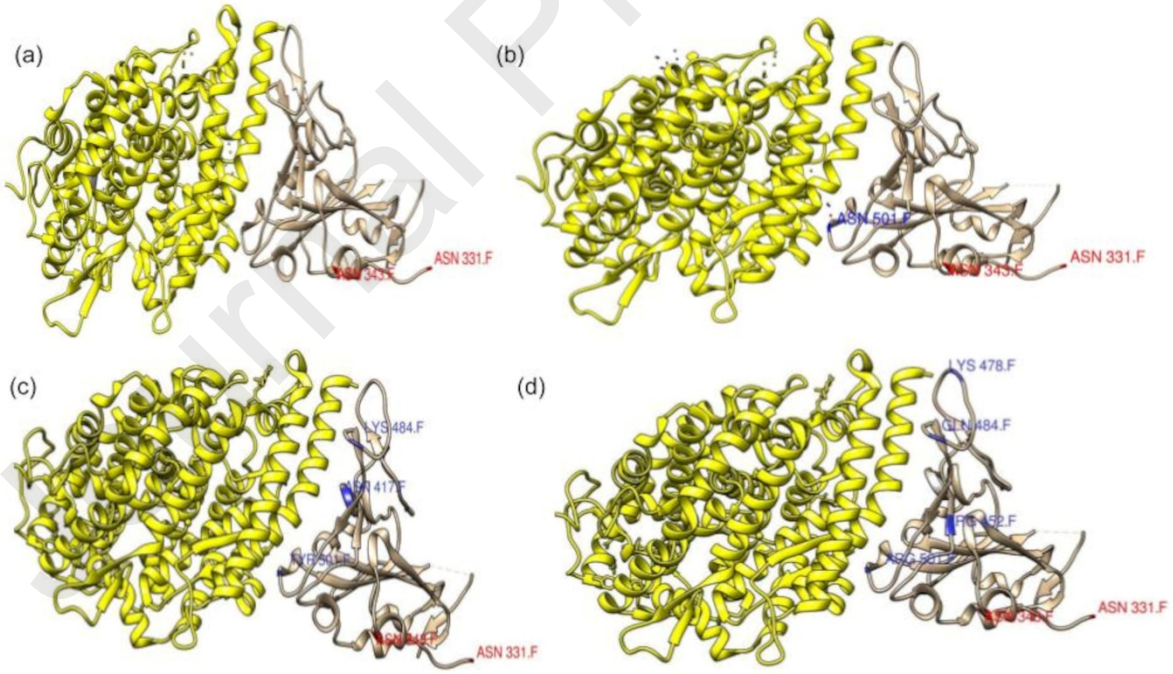

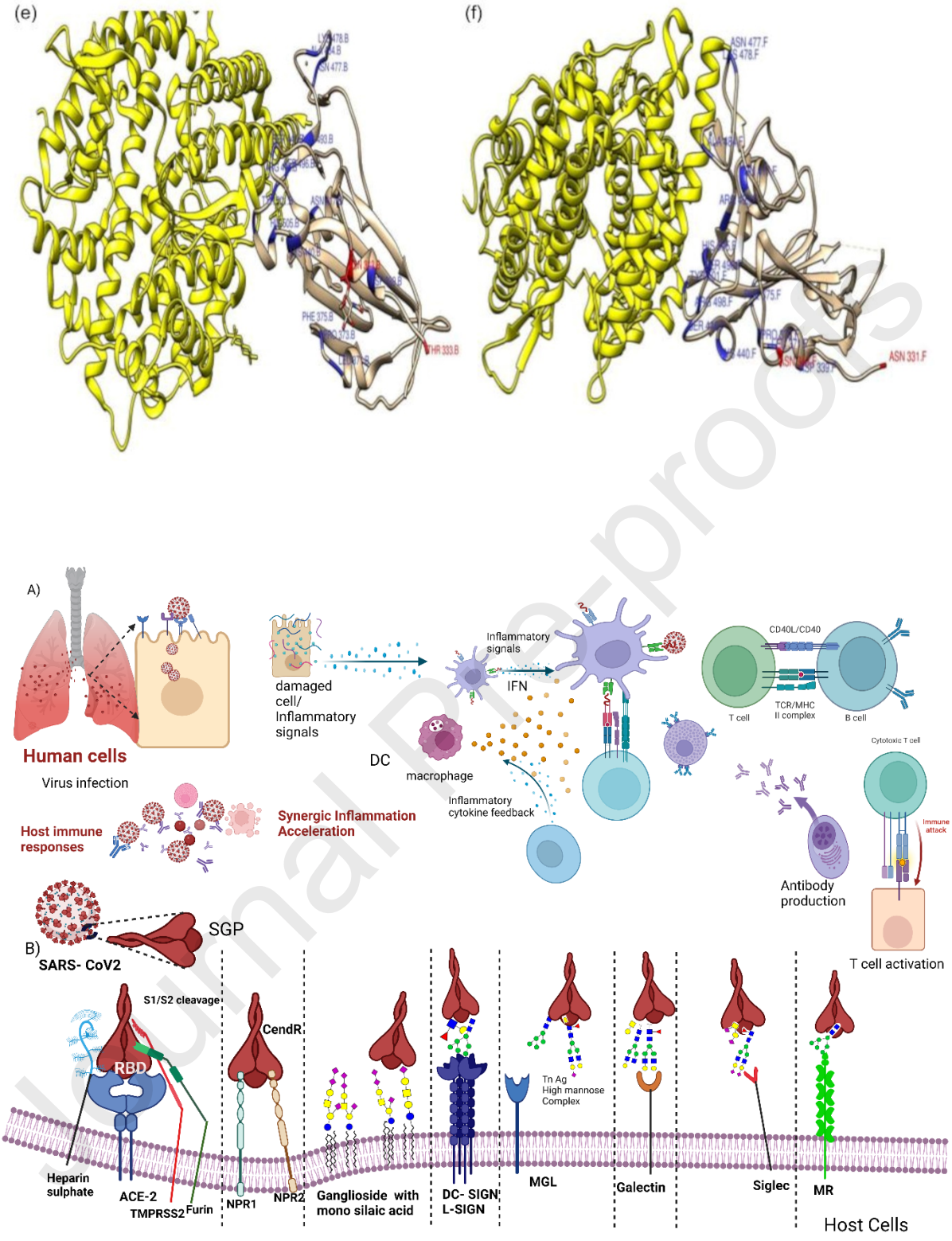

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Parvathy Venugopal: Editing and impoving the draft

Bipin Gopalakrishnan Nair: Editing and impoving the draft

Renuka Suravajhala: Editing the draft, Figure and submission

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

No financial support