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Hypothesis

Potential Role of Galectin-glycan Lattices in SARS-CoV-2 Infection and Pathogenesis: A Hypothesis

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Abstract

Endothelial dysfunction plays a crucial role in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and has recently been proposed to be connected with acute thrombosis, hyper-inflammation, cytokine storm syndrome, immune cell recruitment, platelet aggregation, vasoconstriction and endothelial apoptosis. Importantly, certain mediators and pro-inflammatory cytokines such as galectin (Gal) 1, Gal3 and Gal8 act in a concerted manner through the N- and O-linked glycans located on the SARS-CoV-2 S protein. We hypothesize that the presence of these factors may cause the ACE2 receptor, integrin β1, and CD44 to generate a Gal-glycan lattice on the surface of SARS-CoV-2 virus. This lattice, in addition to endothelial cells (ECs), may not only influence EC behavior and the inflammatory response, but may also induce conformational changes in the viral structure that can facilitate attachment and entry into the ECs. We believe that further basic science research is necessary to elucidate the composition and role of the Gal-glycan lattices in the SARS-CoV-2 infection.

Introduction

Infection with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) occasionally causes endothelial dysfunction, pulmonary vascular changes and cardiovascular complications.[1–7] Emerging data suggest a critical role of endothelial dysfunction in SARS-CoV-2 infection and the connection with acute thrombosis, hyperinflammation, cytokine storm syndrome, immune cell recruitment, platelet aggregation, vasoconstriction and endothelial apoptosis.[1,4–7] Of note, the secretion of pro-inflammatory cytokines, such as interleukin (IL) 1β, IL6, IL10, tumor necrosis factor alpha, and immune cell recruitment, has been shown to lead to endothelial cell (EC) activation.[5–7] Moreover, during SARS-CoV-2 infection, significant changes have been described in endothelial morphology involving the loss of cell-cell contacts or adherents junctions, the separation from the basal lamina, and swelling and apoptosis.[2,4,6,7] However, whether these changes are related to endothelial cell dysfunction continues to be a matter of debate.[1,3,7]

Hypothesis: potential role of Galectin-glycan lattices in SARS-CoV-2 infection and pathogenesis

We hypothesize that, in the presence of certain mediators and pro-inflammatory cytokines, Galectin (Gal) 1, Gal3 and Gal8 act in a concerted manner through the N-and O-linked glycans located on the SARS-CoV-2 S protein. We believe that angiotensin-converting enzyme 2 (ACE2) receptor, integrin β1, and CD44 then generate a Galectin-glycan (Gal-glycan) lattice on the surface of the virus and ECs (Fig. 1). Such Gal-glycan lattice may not only influence the EC behavior and the inflammatory response, but also induce conformational changes in the viral structure that can facili-

Keywords: SARS-CoV-2 infection; Endothelial dysfunction; N-linked glycan; Galectin-glycan lattice.

Abbreviations: ACE2, angiotensin-converting enzyme 2; CRD, carbohydrate-recognition domain; CTD, carboxy terminal domain; EC, endothelial cell; Gal, galectin; Gal-glycan, Galectin-glycan; NTD, N-terminal domain; RBD, receptor binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

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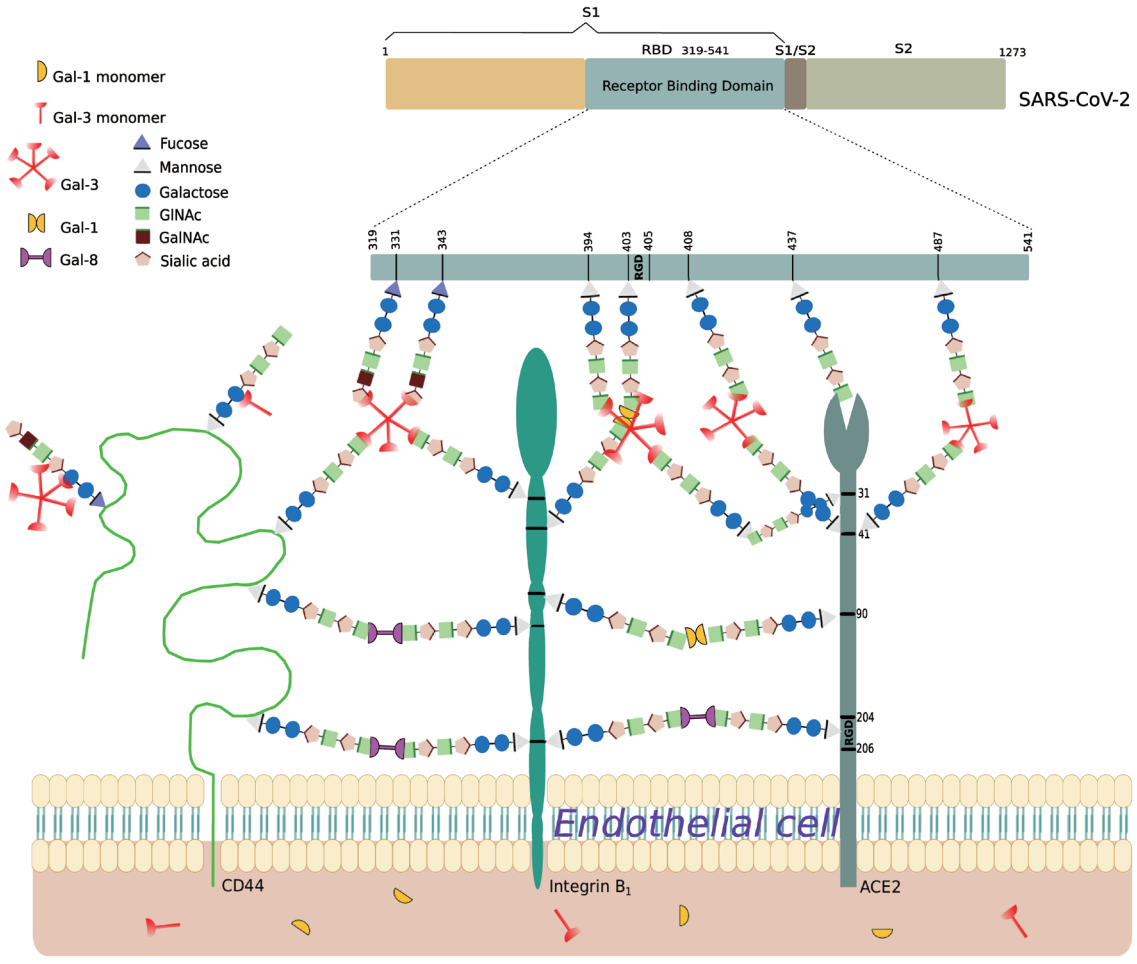

Fig. 1. Schematic representation of the full-length of SARS-CoV-2 spike (S) glycoprotein (aa1-aa1273) showing the S1 and S2 domains, the region binding domain (RBD) and the furin cleavage site (S1/S2). The magnified view shows Gal1, Gal3 and Gal8 forming a Gal-glycan lattice on the SARS-CoV-2 virus and endothelial cell, through the N- and O-linked glycan residues that are located on the RBD domain (N319-N541), ACE2 receptor, integrin β_1, and CD44. The numbers indicate the position of glycans in the sequence. RGD motifs (Arg, Gly, Asp) in RBD (N403-N405) and ACE2 (N204-N206).

tate attachment and entry into the ECs.

Evaluation of the hypothesis

SARS-CoV-2 attachment and entry

Several studies have proposed that SARS-CoV-2 enters ECs by endocytosis through the binding of its trimeric spike (S) glycoprotein to a cellular receptor, which then promotes virus attachment to the surface of targeted cells, membrane fusion and entry.[1,3,4,6] ACE2 is a transmembrane protein widely expressed in ECs,[1,3–6] and is considered the main functional receptor mediating the entrance of SARS-CoV-2 into the host cells. Other proteins on the EC surface, such as transmembrane protease serine-2 (TMPRS-2), sialylated glycans, extracellular matrix metalloproteinase inducer (CD147), the glycoproteins integrin β_1 and CD44, also interact with the N-terminal domain (NTD) and C-terminal domain (CTD) of the SARS-CoV-2 S glycoprotein and may mediate its entry.[3,4] Importantly, studies using angiotensin II receptor blockers suggest that elevated cellular ACE2 expression facilitates the binding of SARS-CoV-2, which is also related to the severity of the infection.[8] However, the functional determinants of SARS-CoV-2 attachment and entrance into target cells still needs to be clarified.

SARS-CoV-2 S glycoprotein structure

The trimeric S glycoprotein is synthesized as a single 1273 amino acid polypeptide chain that is extensively glycosylated and processed by host proteases. Each monomer is composed of two functional subunits or domains (S1 and S2).[8–10] In turn, the S1 domain comprises two different regions: the CTD and the NTD.[8–10] In particular, the CTD mainly binds peptides while NTD binds extracellular sugars.[10] In fact, it has been proposed that the SARS-CoV-2 binds to ACE2 receptors via its CTD domain.[8–12] The S1 domain also contains a receptor binding domain (RBD) and seems to be responsible for initial virus attachment to the host cell. This likely occurs through binding to the ACE2 receptor and subsequently allows for conformational change of the S2 domain, membrane fusion and finally, virus entry.[8–12] The RBD contains a conserved RGD (Arg, Gly, Asp) (N403-N405) motif which seems to mediate the virus attachment by way of integrins,[8,13] Furthermore, at least two O-

glycosylation sites (N331 and N343) exhibit considerable levels of fucosylation and N-acetyl galactosylation (GalNAc).[14] Another characteristic of the SARS-CoV-2 S glycoprotein is the presence of the S1/S2 site that is processed by the cellular protease furin. Importantly, structural evidence has stressed that each monomer of the SARS-CoV-2 S glycoprotein has 22 N-linked glycosylation sites, of which 14-16 seem to be occupied by N- glycans, and at least three or four are predicted to be O-linked glycosylation sites.[5,8,10,14,15] By contrast, ACE2 contains five - seven potential glycosylation sites.[8,12] Nevertheless, a remaining question is whether certain N- and O-linked glycans in the SARS-CoV-2 S glycoprotein also actively influence the process leading to infection.

Glycans and galectins

Glycans are oligosaccharides chain complexes located on the cell surface and that mediate molecular and cellular interactions in biological processes. These processes are mediated via specific signaling pathways and include endocytosis, cell growth, motility, adhesion, autoimmunity, angiogenesis and tumor development.[16] Two types of glycan residues are present in the human glycoproteins: N- and O-linked glycans. The most common N-linked glycans are composed of sialic acid, N-acetylglucosamine (GlcNAc), galactose, and mannose (Man), while the O-linked glycans are composed of N-acetylgalactosamine, (GalNAc), galactose, GlcNAc, and fucose. Regarding the possible role of N-glycans linked to the S glycoprotein trimers at certain sites during the viral process, some studies have proposed that they increase the stability and solubility of the virus, acting as camouflage to evade the host immune response.[8] Interestingly, glycans can also have an indirect effect on virus-host cell interactions with the participation of the soluble lectin family named Gals.[17–19] Gals are a sub-family of highly-conserved glycan-binding proteins that are defined by the presence of one or two carbohydrate recognition domains (CRDs) with an affinity for β-galactoside.[17–19] Such an affinity seems to be determined by the number of glycosylation sites. In general, Gals regulate several physiological and pathological processes, including viral infections, with most influential ones being Gal1, Gal3 and Gal8.[17,19–21] Additionally, studies have shown that ECs synthesize Gal1, Gal3 and Gal8, and that EC activation can be induced by cytokines that are released at the site of inflammation. This synthesis stimulates Gal expression and induces changes in cellular localization through the activation of specific EC surface glycoproteins.[19,22–24]

Gal1 is a mammalian lectin with a conserved CRD that has an affinity for disaccharides containing galactose and GlNAc present in N- and O-linked glycans. Gal1 can form homodimers through its C-terminal-domain, which bind and cross-link specific targets containing N- and O-glycans on the cell surface. Gal1 can additionally bind to extracellular milieu which gives rise to the formation of various molecular complexes.[17–19,22] Furthermore, it is well established that the expression of Gal1 is increased during EC activation.[19,22,24] Gal3 is the only chimera-type Gal and consists of a C-terminal domain that binds to specific N- and O-glycan ligands and an N-terminal domain. This binding facilitates its pentamerization and generation of Gal-glycan lattices on the cell surface and to extracellular milieu that regulate, through specific signaling pathways, several biological functions such as cell-cell, cell-matrix adhesion, proliferation, growth, differentiation, migration, inflammation, immune response, fibrosis, apoptosis and tumor development.[18,19,22,23] Gal8 is a tandem-repeat type of Gal that possesses two CRDs joined by a linker peptide. Gal8 appears to be one of the most conspicuous Gals detected in ECs, and plays an important role in the control of EC migration, capillary tube formation and *in vivo* angiogenesis.[22,24] Similar to Gal1 and Gal3, Gal8 interacts with the N- and/or O-linked glycan residues of the cell surface glycoproteins integrin β_1 and CD44, which are also recognized as binding partners of these Gals that regulate cell attachment, spreading and migration.[22,25]

Galectins, the SARS-CoV-2 S glycoprotein, ACE2 receptor, integrin β_1, and CD44

Although glycans and Gals seem to be important to the virus-host cell interaction,[19–21] to the best of our knowledge, there are no reports on the generation and role of a Gal-glycan lattice in the SARS-CoV-2 infection. There is, however, information, albeit limited, regarding the function of Gal3 in coronavirus disease 2019 (COVID-19). For instance, an important role of Gal3 in COVID-19 in terms of regulating the inflammatory response, fibrosis and infection progression has been suggested.[26] Moreover, it has been proposed that Gal3 may augment the cytokine storm syndrome described in severe COVID-19 cases.[27] Also, high levels of Gal3 have been detected in the serum of patients suffering from severe COVID-19 infection.[27] As for Gal1, it has been shown that this Gal can recognize N-glycans on certain viruses such as HIV, herpes simple virus, dengue virus and Nipah virus, which significantly increases the rate of viral attachment to and entry into the host cell.[19–21] By contrast, Gal8 has been suggested to stimulate the secretion of pro-inflammatory cytokines from ECs and is thus involved in the regulation of the immune system.[19–21] Nevertheless, whether these Gals are able to generate a Gal-glycan lattice in SARS-CoV-2 infection should be further explored.

It is currently known that Gal1, Gal3, and Gal8 contribute to the formation of the Gal-glycan lattice on the surface of ECs and immune cells through their binding partners that include integrin β_1 and CD44, and this aid in regulating the behavior of these cells.[22,23] In the SARS-CoV-2 context, some studies have proposed that integrins bind to a conserved RGD motif (Arg, Gly, Asp) present in the RBD domain of the S glycoprotein, and mediate virus attachment and facilitate entrance into the host cell.[13,28] Other studies have suggested that integrins also bind to ACE2 in a conserved RGD motif, which regulates cell proliferation and survival.[29] Despite these findings, not much is known about the participation of integrins and CD44 in the generation of Gal-glycan lattices and the potential implications of such lattices in the pathogenesis of SARS-CoV-2 infection.

Future directions

The evidence supporting our hypothesis highlights the importance of Gals, particularly Gal-glycan lattices, in SARS-CoV-2 infection. In this sense, studies should be expanded to examining the use of specific small-molecule glycans or synthetic inhibitors of Gal-glycan interactions, Gal antagonists and truncate forms in cell line cultures as therapeutic agents in the treatment or prevention of COVID-19 infection. Such investigations should be explored with the aim of limiting viral entry and modulating the immune response against foreign entities.

Conclusions

Finally, we believe that future basic science research will be neces-

sary to establish the composition and role of the Gal-glycan lattices and to understand how SARS-CoV-2 attaches to and enters ECs. Such work will likely also bring forward research programs investigating the role of specific inhibitors of Gal3, ACE2 receptors, and integrins in COVID-19 infection.[6,20,26,27,29]

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Conflict of interest

The authors declare no potential conflict of interest.

Author contributions

Proposed the hypothesis and wrote the paper (EA), did the literature search (LMC), constructed the figure (AS). All authors revised and approved the final version of the manuscript.

References

[1] Vargas Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, *et al*. Endothelial cell infection and endotheliitis in COVID-19. Lancet 2020;395(10234):1417–1418. doi:10.1016/S0140-6736(20)30937-5.
[2] Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, *et al*. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med 2020;383(2):120–128. doi:10.1056/NEJMoa2015432.
[3] Jung F, Krüger-Genge A, Franke RP, Hufert F, Kupper JH. COVID-19 and the endothelium. Clin Hemorheol Microcirc 2020;75(1):7–11. doi:10.3233/CH-209007.
[4] Huertas A, Montani D, Savale L, Pichon J, Tu L, Parent F, *et al*. Endothelial cell dysfunction: a major player in SARS-CoV-2 infection (COVID-19)? Eur Resp J 2020;56(1):2001634. doi:10.1183/13993003.01634-2020.
[5] Pons S, Fodil S, Azoulay E, Zafrani L. The vascular endothelium: the cornerstone of organ dysfunction in severe SARS-CoV-2 infection. Crit Care 2020;24(1):353. doi:10.1186/s13054-020-03062-7.
[6] Evans PC, Rainger GE, Mason J, Guzik TJ, Osto E, Stamataki Z, *et al*. Endothelial dysfunction in COVID-19: a position paper of the ESC Working Group for Atherosclerosis and Vascular Biology, and the ESC Council of Basic Cardiovascular Science. Cardiovasc Res 2020;116(14):2177–2184. doi:10.1093/cvr/cvaa230.
[7] Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. Eur Heart J 2020;41(32):3038–3044. doi:10.1093/eurheartj/ehaa623.
[8] Chaterjee SK, Saha S, Muñoz MNM. Molecular pathogenesis, immunopathogenesis and novel therapeutic strategy against COVID-19. Front Mol Biosci 2020;7:196. doi:10.3389/fmolb.2020.00196.
[9] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181(2):281–292.e6. doi:10.1016/j.cell.2020.02.058.
[10] Casalino L, Gaieb Z, Goldsmith JA, Hjorth CK, Dommer AC, Harbison MA, *et al*. Beyond shielding: The roles of glycans in the SARS-CoV-2 spike protein. ACS Cent Sci 2020;6(10):1722–1734. doi:10.1021/acscentsci.0c01056.
[11] Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020;367(6485):1444–1448. doi:10.1126/science.abb2762.
[12] Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, *et al*. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020;581(7807):221–224. doi:10.1038/s41586-020-2179-y.
[13] Sigrist CJ, Bridge A, Le Mercier P. A potential role for integrins in host cell entry by SARS-CoV-2. Antiviral Res 2020;177:104759. doi:10.1016/j.antiviral.2020.104759.
[14] Lenza MP, Oyenarte I, Diercks T, Quintana JI, Gimeno A, Coelho H, *et al*. Structural characterization of N-linked glycans in the receptor binding domain of the SARS-CoV-2 spike protein and their interactions with human lectins. Angew Chem Int Ed Engl 2020;59(52):23763–23771. doi:10.1002/anie.202011015.
[15] Shajahan A, Supekar NT, Gleinich AS, Azadi P. Deducing the N- and O-glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2. Glycobiology 2020;30(12):981–988. doi:10.1093/glycob/cwaa042.
[16] Varki A. Biological roles of glycans. Glycobiology 2017;27(1):3–49. doi:10.1093/glycob/cww086.
[17] Liu FT, Rabinovich GA. Galectins: Regulators of acute and chronic inflammation. Ann N Y Acad Sci 2010;1183:158–182. doi:10.1111/j.1749-6632.2009.05131.x.
[18] Nabi IR, Shankar J, Dennis JW. The galectin lattice at a glance. J Cell Sci 2015;128(13):2213–2219. doi:10.1242/jcs.151159.
[19] Thiemann S, Baum LG. Galectins and immune responses-Just how do they do those things they do? Annu Rev Immunol 2016;34:243–264. doi:10.1146/annurev-immunol-041015-055402.
[20] Machala EA, McSharry BP, Rouse BT, Abendroth A, Slobedma B. Gal power: the diverse roles of galectins in regulating viral infections. J Gen Virol 2019;100(3):333–349. doi:10.1099/jgv.0.001208.
[21] Wang WH, Lin CY, Chang MR, Urbina AN, Assavalapsakul W, Thitithanyanont A, *et al*. The role of galectins in virus infection – A systemic literature review. J Microbiol Immunol Infect 2020;53(6):925–935. doi:10.1016/j.jmii.2019.09.005.
[22] Elola MT, Ferragut F, Méndez-Huergo SP, Crocci DO, Bracalante C, Rabinovich GA. Galectins: multitask signaling molecules linking fibroblast, endothelial and immune cell programs in the tumor microenvironment. Cell Immunol 2018;333:34–45. doi:10.1016/j.cellimm.2018.03.008.
[23] Arciniegas E, Carrillo LM, Rojas H, Ramírez R, Chopite M. Galectin-1 and galectin-3 and their potential binding partners in the dermal thickening of keloid tissues. Am J Dermatopathol 2019;41(3):193–204. doi:10.1097/DAD.0000000000001284.
[24] Thijssen VL, Rabinovich GA, Griffioen AW. Vascular galectins: regulators of tumor progression and targets for cancer therapy. Cytokine Growth Factor Rev 2013;24(6):547–558. doi:10.1016/j.cytogfr.2013.07.003.
[25] Eshkar Sebban L, Ronen D, Levartovsky D, Elkayam O, Caspi D, Aamar S, *et al*. The involvement of CD44 and its novel ligand galectin-8 in apoptotic regulation of autoimmune inflammation. J Immunol 2007;179(2):1225–1235. doi:10.4049/jimmunol.179.2.1225.
[26] García-Revilla J, Delerborg T, Venero JL, Boza-Serrano A. Hyperinflammation and fibrosis in severe COVID-19 patients: Galectin-3, a target molecule to consider. Front Immunol 2020;11:2069. doi:10.3389/fimmu.2020.02069.
[27] Caniglia JL, Asuthkar S, Tsung AJ, Guda MR, Velpula KK. Immunopathology of galectin-3: an increasingly promising target in COVID-19. F1000Res 2020;9:1078. doi:10.12688/f1000research.25979.2.
[28] Yan S, Sun H, Bu X, Wan G. New strategy for COVID-19: An evolutionary role for RGD motif in SARS-CoV-2 and potential inhibitors for virus infection. Front Pharmacol 2020;11:912. doi:10.3389/fphar.2020.00912.
[29] Pirone L, Del Gatto A, Di Gaetano S, Saviano M, Capasso D, Zaccaro L, *et al*. A multi-targeting approach to fight SARS-CoV-2 attachment. Front Mol Biosci 2020;7:186. doi:10.3389/fmolb.2020.00186.