Immunopathology of galectin-3: an increasingly promising target in COVID-19 [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract
The pandemic brought on by the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) has become a global health crisis, with over 22 million confirmed cases and 777,000 fatalities due to coronavirus disease 2019 (COVID-19) reported worldwide. The major cause of fatality in infected patients, now referred to as the “Cytokine Storm Syndrome” (CSS), is a direct result of aberrant immune activation following SARS-CoV2 infection and results in excess release of inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor α (TNF-α), and IL-6, by macrophages, monocytes, and dendritic cells. Single cell analysis has also shown significantly elevated levels of galectin 3 (Gal-3) in macrophages, monocytes, and dendritic cells in patients with severe COVID-19 as compared to mild disease. Inhibition of Gal-3 reduces the release of IL-1, IL-6, and TNF-α from macrophages in vitro, and as such may hold promise in reducing the incidence of CSS. In addition, Gal-3 inhibition shows promise in reducing transforming growth factor β (TGF-β) mediated pulmonary fibrosis, likely to be a major consequence in survivors of severe COVID-19. Finally, a key domain in the spike protein of SARS-CoV2 has been shown to bind N-acetylneuraminic acid (Neu5Ac), a process that may be essential to cell entry by the virus. This Neu5Ac-binding domain shares striking morphological, sequence, and functional similarities with human Gal-3. Here we provide an updated review of the literature linking Gal-3 to COVID-19 pathogenesis. Dually targeting galectins and the Neu5Ac-binding domain of SARS-CoV2 shows tentative promise in several stages of the disease: preventing viral entry, modulating the host immune response, and reducing the post-infectious incidence of pulmonary fibrosis.
Introduction
Galectin 3 (Gal-3) is an animal lectin that exhibits pleiotropic effects throughout the body, including the modulation of apoptosis, cell migration and adhesion, angiogenesis, tumorigenesis, and post-injury remodeling (Chen & Kuo, 2016; Elola et al., 2018; Nangia-Makker et al., 2018). Recent discoveries have begun to shed light on its role in viral infections as well (Wang et al., 2019). In particular, Gal-3 has been shown during infection to induce a dysregulated pattern expression of pro-inflammatory cytokine expression via the JAK/STAT1, ERK, and AKT signaling pathways (Nita-Lazar et al., 2015). The cytokine profile observed includes tumor necrosis factor α (TNFα), interleukin (IL)-1β, and IL-6, among others (Nita-Lazar et al., 2015). Gal-3 is also a known agonist of toll like receptor 4 (TLR4) and nuclear factor kappa beta (NF-kB) dependent pathways, which are well characterized and potent inducers of inflammation during infection (Yip et al., 2017; Zhou et al., 2018). Patients suffering from severe coronavirus disease 2019 (COVID-19) show highly elevated levels of Gal-3, TNFα, IL-1β, and IL-6, as compared to those with moderate disease (De Biasi et al., 2020; Wang et al., 2020a). Inhibition of Gal-3 significantly reduces the levels of these cytokines, and so may show promise in reducing inflammatory sequelae associated with COVID-19 (De Biasi et al., 2020; Kalfaoglu et al., 2020; Liu et al., 2020).

The continued lack of an effective standard of care for treating patients with COVID-19 has brought on an urgent need to identify effective therapies. In a prior review article, we had discussed promising indications for Gal-3 targeted therapy in the treatment of COVID-19, with the goal of inspiring further research on the topic (Canighia et al., 2020). In recent months, however, a substantial amount of new evidence has emerged that further links Gal-3 to severe COVID-19 infection. As such, the authors see a need to achieve two aims in this review: highlighting novel discoveries to expand upon previously discussed treatment indications, and to detail a further potential role for anti-galectin therapy in reducing post-infectious pulmonary fibrosis. This article may be particularly useful for immunologists studying COVID-19, as well as any researchers with a structural or functional focus on galectins.

SARS-CoV2: host cell attachment and entry
A critical step prior to viral infection is the entry of the virus into host cells, a process that in severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) is mediated by the S1 subunit of the spike protein (Blaas, 2016; Zhai et al., 2020). Within coronaviridae, it is commonplace to refer to the S1 protein as consisting of two distinct regions: the C-terminal domain (CTD) and N-terminal domain (NTD) (Li, 2016). In most cases, the CTD binds peptide receptors and the NTD binds sugar receptors (Li, 2016). The main entry mechanism of SARS-CoV2 has been shown to be via the CTD binding to angiotensin converting enzyme receptor 2 (ACE2) receptors (Wang et al., 2020b). Until recently, the role of the NTD has been largely overlooked. A study from Baker et al. has shown evidence that SARS-CoV2 also binds N-acetylmuramic acid (Neu5Ac), with this interaction being mediated by the NTD of the S1 subunit (Baker et al., 2020). This is the first in vitro evidence of this occurring, although several prior bioinformatics and modeling studies have hypothesized that a Neu5Ac binding site exists, with one suggesting its affinity for Neu5Ac (0.88) is only slightly lower than that of influenza hemagglutinin (0.94) (Alban et al., 2020; Behloul et al., 2020; Fantini et al., 2020; Kim, 2020; Milanetti et al., 2020; Robson, 2020). Binding of sialic acids by the NTD is the main entry mechanism in several other coronaviruses known to infect humans, most notably members of the bovine coronavirus family (Li, 2015). Additionally, the closely related middle eastern respiratory syndrome coronavirus (MERS-CoV) has been shown to exhibit a dual attachment model similar to SARS-CoV2, where the CTD binds a peptide receptor and the NTD binds sialic acids (Li et al., 2017). Depletion of sialic acids with neuraminidase inhibitors prevented MERS-CoV infection of Calu-3 human airway cells, indicating that NTD-targeted therapies may be effective in preventing cell entry by coronaviruses possessing this function (Li et al., 2017). The dual mechanism by which SARS-CoV2 may enter host cells is seen in Figure 1.

The binding of Neu5Ac may also explain the greater infectivity of SARS-CoV2 as compared to SARS-CoV (Alban et al., 2020). While the CTD of SARS-CoV2 has been shown to exhibit higher affinity for ACE2 receptors than that of SARS-CoV, this is likely insufficient to fully explain the marked disparity in transmissibility (Tai et al., 2020). The NTD of SARS-CoV2 has been rigorously analyzed and compared to both human galectins and the NTD of other coronaviruses (Behloul et al., 2020). Behloul et al. found that while SARS-CoV2 and SARS-CoV share 74.75% similarity in the CTD, they exhibit just 52.69% similarity in the NTD region (Bebloul et al., 2020). This is particularly noteworthy when viewed together with the findings that despite SARS-CoV2 being able to bind Neu5Ac in vitro, the same domain on SARS-CoV did not exhibit this ability (Baker et al., 2020). Modeling studies comparing the NTD of SARS-CoV2 and SARS-CoV have led to the same conclusion (Bebloul et al., 2020). The far greater abundance of Neu5Ac in the human body as compared to ACE2 receptors, particularly at common viral entry points such as the nasopharynx and oral mucosa, may explain the high transmissibility of SARS-CoV2 (Barnard et al., 2019).

Several studies to date have referred to the “galectin fold” present on the NTD of coronaviruses (Behloul et al., 2020; Li, 2016; Li et al., 2017; Peng et al., 2011; Peng et al., 2012; Tortorici et al., 2019). The structures are so similar, in fact, that it is hypothesized that coronaviruses incorporated a host galectin gene into their genome (and then the NTD) at some point in their evolution (Li, 2015). Structural analysis comparing the SARS-CoV2 NTD to Gal-3 resulted in a Z-score of 6 (p < 0.00001), indicating a high degree of similarity between the structures (Bebloul et al., 2020). In fact, human Gal-3 was shown to be equally similar to SARS-CoV2 NTD as the NTD of NL63-CoV and infectious bronchitis coronavirus, accounting for both sequence and structure (Bebloul et al., 2020). Given the high degree of structural and promising sequence similarity (12%) of the NTD with Gal-3, it may be…
possible that existing Gal-3 inhibitors possess dual-binding capabilities (Behloul et al., 2020). Such a mechanism shows promise in reducing viral entry to host cells (Milanetti et al., 2020).

**Gal-3 in severe infection: promoting immunologic sequelae of COVID-19**

The major cause of death in patients infected with SARS-CoV and MERS-CoV infection was found to be the “Cytokine Storm Syndrome” (CSS), and this is likely to be the case in COVID-19 as well (Channappanavar & Perlman, 2017; Zhang et al., 2020). CSS develops due to hyper-activation of macrophages, monocytes, and dendritic cells, which are stimulated to release a variety of inflammatory mediators including IL-1, IL-6, and TNF-α (Zhang et al., 2020). This in turn leads to systemic organ dysfunction that may result in death (England et al., 2020). Notably, a study of nearly 4,000 patients has found the levels of IL-1, IL-6, and TNF-α to be significantly elevated in the sera of patients suffering from severe COVID-19 as compared to those with mild disease (Wang et al., 2020a). This data speaks to the urgency of identifying therapeutics to reduce the incidence of CSS (Wang et al., 2020a).

There is a plethora of evidence that makes Gal-3 a promising target to achieve this aim. First, the most concerning sequelae of CSS is evolution to acute respiratory distress syndrome (ARDS), a condition which often leads to respiratory failure despite proactive measures such as mechanical ventilation and intubation (Vabret et al., 2020). Elevated serum levels of Gal-3 are significantly associated with worse outcomes and lower survival in patients suffering from ARDS (Xu et al., 2017). Additionally, significantly elevated levels of Gal-3 have been shown in the serum of patients suffering from severe COVID-19 as compared to those with mild disease (De Biasi et al., 2020). On a cellular level, Gal-3 was shown to be most elevated in immune cells during severe COVID-19 (Kalfaoglu et al., 2020). The highest levels of Gal-3 were seen in infected macrophages, monocytes, and dendritic cells, the very cells responsible for initiating CSS (Liu et al., 2020). A pathway through which Gal-3 may contribute to the development of CSS is detailed in Figure 2.

Several studies to date have shown the effects of anti-Gal-3 therapy on cytokine release. Significant reductions in IL-1, IL-6, and TNF-α secretion by dendritic cells has been observed upon silencing of Gal-3 (Chen et al., 2015). In models of

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**Figure 1. A dual attachment model for SARS-CoV2.** Evidence has shown that a pocket in the NTD of SARS-CoV2 is capable of binding N-acetyleneuraminic acid (Neu5Ac). This strongly supports a dual attachment model for SARS-CoV2, where NTD-Neu5Ac interactions facilitate initial host cell recognition by the virus and stabilize its entry via ACE2 receptors.
traumatic brain injury and spinal cord injury, treatment with anti-Gal-3 antibodies and the Gal-3 inhibitor GB1107, respectively, both led to significant reductions in the systemic levels of IL-1, IL-6, and TNF-α (Ren et al., 2019; Yip et al., 2017). Lastly, in mice infected with H5N1 influenza virus, Gal-3 K/ O led to a significant reduction of IL-1ß secretion by macrophages and improved survival rate as compared to controls (Chen et al., 2018). These findings are due to Gal-3’s known role as an alarmin of the innate immune system, triggering the release of inflammatory cytokine, such as TNF-α and IL-6 from monocyte-derived cells during infection or other inflammatory insults (Mishra et al., 2013; Yip et al., 2017). The enhanced secretion of cytokines likely occurs through TLR4/NF-kB mediated pathways (Yip et al., 2017; Zhou et al., 2018). With all this information taken together, Gal-3 inhibition shows promise in reducing the incidence and symptoms of CSS.

**Gal-3 post-infection: pathologic fibrosis**

It is well known that persistent viral infections are a risk factor for the subsequent development of pulmonary fibrosis (Sheng et al., 2020). A study found that tests for SARS-CoV2 RNA in the serum of infected individuals did not become negative until a median of 24 days post-symptom onset, with some individuals remaining positive even greater than a month from the beginning of symptoms (Gombar et al., 2020). This indicates that for some, COVID-19 infection may run a particularly long course. Findings such as this have led to the question of whether or not anti-fibrotic therapy would be beneficial for such patients (George et al., 2020).

In SARS-CoV infection, particularly in patients who suffered from ARDS, marked pulmonary fibrosis was found in a cohort of patients following prolonged infection (Ye et al., 2007). Though long term outcomes remain to be seen, lung tissue in the acute phase of COVID-19 shows similar changes (Xu et al., 2020a). Following a 24 hour incubation of SARS-CoV2, human airway cells showed upregulation of ACE2, vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF), fibronectin (FN), and transforming growth factor β (TGF-β), a molecular signature highly similar to that of patients with diagnosed pulmonary fibrosis (Xu et al., 2020a). It is believed that a large number of COVID-19 patients will

![Figure 2. Gal-3 may amplify the cytokine storm syndrome associated with severe COVID-19. During severe SARS-CoV2 infection, increased concentrations of Gal-3 are observed in macrophages, monocytes, and dendritic cells. When secreted, Gal-3 can then agonize TLR4 receptors on their surfaces and induce the release of inflammatory cytokines such as IL-1, IL-6, and TNF-α. This process also results in the secretion of further Gal-3, resulting in a positive feedback loop that may contribute to the development of CSS.](https://example.com/figure2.png)
go on to develop pulmonary fibrosis, and that these changes are mediated by a number of cytokines including TGF-β, IL-1, IL-6, and TNF-α (Delpino & Quarleri, 2020).

The role of Gal-3 as a mediator of lung fibrosis has long been studied since the discovery that its levels are elevated in alveolar macrophages following lung injury (Kasper & Hughes, 1996; Nishi et al., 2007). Higher levels of Gal-3 have now been extensively associated with the development of restrictive lung diseases (Ho et al., 2016). Following cellular stress, the secretion of Gal-3 by macrophages upregulates TGF-β receptors on fibroblasts and myofibroblasts (Henderson et al., 2008). This in turn activates these cells, initiating the formation of granulation tissue (via collagen deposition) that is eventually remodeled to a fibrous scar (Henderson et al., 2008; Mackinnon et al., 2012). This Gal-3 mediated pathway is widespread throughout the body and fundamental to the development of fibrotic change in the liver, kidneys, and heart as well (Hara et al., 2020). Gal-3 mediated fibrosis often has deleterious effects; for example, pathologic scar formation is the likely explanation for serum Gal-3’s utility as an independent predictor of mortality and heart failure post-myocardial infarction (Asleh et al., 2019). The mechanism by which Gal-3 may contribute to post-infectious pulmonary fibrosis in COVID-19 patients can be seen in Figure 3.

Gal-3 inhibitors show promise in limiting fibrotic change following lung injury. In a model of adenovirus induced lung injury, Gal-3 K/O mice showed significant reductions in lung fibrosis and β-catenin activation, indicating the beneficial effects were mediated via interruption of TGF-β signaling (Mackinnon et al., 2012). Treatment with the drug TD139 showed significant reductions in these parameters as well following bleomycin-induced pulmonary fibrosis (Mackinnon et al., 2012). This drug (now referred to as GB0139) was well tolerated in phase I/IIa trials in the treatment of idiopathic pulmonary fibrosis (IPF) and is now in phase IIb trials (Saito et al., 2019). An additional indication for this drug may be in reducing the post-viral development of pulmonary fibrosis (Mackinnon et al., 2012). The drug TD139 has recently begun phase II trials for the

Figure 3. Gal-3 contributes to a pro-fibrotic microenvironment in COVID-19. During SARS-CoV2 infection, transcriptional upregulation of VEGF, TGF-β, and fibronectin (FN) is seen in the pulmonary epithelium, creating a pro-fibrotic microenvironment. Secretion of Gal-3 by macrophages contributes to fibrosis by increasing the expression of TGF-β receptors on the surface of fibroblasts. The fibroblasts and myofibroblasts are then activated by TGF-β mediated signaling, stimulating the deposition of extracellular matrix and collagen that leads to fibrotic damage. Cytokines induced by Gal-3 expression such as IL-1, IL-6, and TNF-α further accelerate this process.
A key domain in the spike protein exhibits a high degree of morphological and sequence similarity to human Gal-3 (Belioul et al., 2020). This NTD has been shown to bind Neu5Ac in vitro, an interaction that likely explains the high infectivity of SARS-CoV2 and may be essential for cell entry (Alban et al., 2020; Barnard et al., 2019; Baker et al., 2020). Inhibitors of Gal-3 that target regions of structural overlap with the NTD may possess dual binding capabilities, exhibiting a novel mechanism by which to inhibit viral entry (Milanetti et al., 2020).

Lastly, pulmonary fibrosis has been observed following SARS-CoV infection and is likely to be a major complication in survivors of COVID-19 that is cytokine-mediated (Delpino & Quarleri, 2020; Xu et al., 2020b; Ye et al., 2007). Among other mediators, elevated levels of TGF-β have been observed following SARS-CoV2 infection (Xu et al., 2020a). Gal-3 secreted by macrophages during injury promotes the upregulation of TGF-β receptors, leading to fibroblast activation and collagen deposition (Delpino & Quarleri, 2020). Gal-3 inhibition has been shown to reduce adenovirus-induced lung fibrosis, and an inhibitor is currently in Phase Ib clinical trials for IPF treatment (Mackinnon et al., 2012; Saito et al., 2019). The indications for targeting Gal-3 in the treatment of COVID-19 are widespread. Processes directly mediated or affected by Gal-3 have been shown to be deleterious in several stages of the disease process. As such, Gal-3 represents a highly promising target for COVID-19 treatment that should urgently be investigated.

### Literature search methodology

#### Eligibility criteria

This review consists of original studies that provided information about SARS-CoV2, Gal-3, or Gal-3 inhibitors. Compiled results from both *in vivo*, *in vitro*, and clinical studies were used for analysis. Studies with only an abstract or no full-text available were excluded from the review.

#### Search methodology

To retrieve primary literature, electronic searches were performed on PubMed and Google Scholar. A list of search terms can be seen in Table 1.

#### Risk of bias

To minimize the risk of error, all authors involved assessed the cited studies for quality. To discuss important claims in the article, including that SARS-CoV2 binds sialic acids with the S1-NTD, that Gal-3 is upregulated in human immune cells, and Gal-3 inhibitors’ ability to reduce fibrosis, multiple sources were included. Additionally, the use of open-ended searches ensured that an accurate profile of results was obtained on the topics discussed.

#### Data availability

No data are associated with this article.

### Acknowledgments

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Robson B: "Bioinformatics studies on a function of the SARS-CoV-2 spike glycoprotein as the binding of host sialic acid glycans." Comput Biol Med. 2020; 122: 103849.

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- The review article is clear, concise, and well structured.
- The article highlights the novel discoveries by linking the pathogenesis of COVID-19 with immunopathologic effects of Gal-3. Dual targeting of Neu5Ac-binding domain of SARS-CoV2 and galectin-3 using galectin-3 inhibitors could be a very effective approach in reducing the spread, cytokine storm, and post-infection pulmonary fibrosis.
- The quality of the figures in the article is good and clearly explains the concept discussed.
- The authors are requested to address the following specific comments mentioned below:
  1) The authors have mentioned Gal-3 inhibitors could target Neu5Ac-binding domain, thereby reducing viral entry to host cells. Since ACE2 receptors serve as the main entry mechanism of SARS-CoV2, To what extent Gal-3 inhibitors alone can provide mitigatory effects?. Also, could ACE2 inhibitors be used as adjuvants along with Gal-3 inhibitors?. What are the possibilities?. These need to be explained.
  2) The authors are advised to cite the article Garcia-Revilla, J. et al., 2020 in their manuscript. The article discusses similar concepts mentioned in the current review by the authors. Therefore, citing it could provide more support to the authors claim.
- Overall the article is well-written and recommended for publication once the minor corrections have been addressed.

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Talia H Swartz
Division of Infectious Diseases, Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA

The authors here describe the role of Galectin 3 in mediating inflammatory cytokine signaling as a possible source of disease pathogenesis in SARS-CoV-2 infection. The review is well written and describes the relevant literature supporting the role of Gal-3 in COVID-19.

The following suggestions would improve the strength of the work:

- The authors have published a similar article entitled: A potential role for Galectin-3 inhibitors in the treatment of COVID-19 [ref-1]; the title of this current article should clearly reflect how these two works are non-overlapping; the authors describe that this is an update of the prior work.

- The authors write: “Recent discoveries have begun to shed light on its role in viral infections (Wang et al. 2019) but should go into further detail about what role(s) it plays; there is a literature on HIV (Wang 2014, Okamoto 2019, Fogel 1999).

- The introduction provides very little information about Galectin 3 besides that it is an animal lectin and exerts pleiotropic effects. Some more description should be provided as to the function of this molecule, its tissue expression, and any literature about epigenetics as it pertains to infection and inflammation.

- On page 3, the authors state: “Several studies to date have referred to the “galectin fold”
present on the NTD of coronaviruses (Behloul et al., 2020; Li, 2016; Li et al., 2017; Peng et al., 2011; Peng et al., 2012; Tortorici et al., 2019). The structures are so similar, in fact, that it is hypothesized that coronaviruses incorporated a host galectin gene into their genome (and then the NTD) at some point in their evolution (Li, 2015). The structures of ‘what’ are so similar? NTD to Gal-3? This should be more explicitly defined. This should refer back to Figure 1 in Caniglia PeerJ 2020.

○ On page 4, the authors state “Notably, a study of nearly 4,000 patients has found the levels of IL-1, IL-6, and TNF-α to be significantly elevated in the sera of patients suffering from severe COVID-19 as compared to those with mild disease (Wang et al., 2020a).” The authors should additionally cite Del Valle et al. Nature Medicine 2020 that noted similar findings in 1500 patients.²

○ Figure 1 does not add richly to this work and perhaps could be a panel combined with Figure 2.

○ The authors state “Several studies to date have shown the effects of anti-Gal-3 therapy on cytokine release.” These studied should be cited.

○ Figure 2 figure legend should address the tissue sites where Gal-3 is produced in macrophages, monocytes, and dendritic cells. Is it lung? Plasma?

○ The authors note on p. 5 “A study found that tests for SARS-CoV2 RNA in the serum of infected individuals did not become negative until a median of 24 days post-symptom onset, with some individuals remaining positive even greater than a month from the beginning of symptoms (Gombar et al., 2020). This indicates that for some, COVID-19 infection may run a particularly long course. Findings such as this have led to the question of whether or not anti fibrotic therapy would be beneficial for such patients (George et al., 2020).” The persistence of SARS-CoV-2 RNA should not be equated with replication competent virus; there is significant literature to suggest residual nucleic acid that does not represent infectious virus. This should not be equated with long term infection or increased risk of fibrotic disease and these patients should not be treated with anti-fibrotic therapy for that reason. Severe lung injury from ARDS would be much more plausible an explanation for fibrotic lung disease than persistent SARS-CoV2 RNA.

References

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** SARS-CoV-2 infection, COVID-19, viral pathogenesis, inflammatory signaling

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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