REVIEW

REVISED Immunopathology of galectin-3: an increasingly promising target in COVID-19 [version 2; peer review: 2 approved]

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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 ß (TGF-ß) 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.

Open Peer Review

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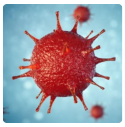
Keywords
COVID-19, galectin, cytokines; ARDS, fibrosis, sialic acid, galectin-3



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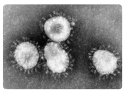

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REVISED **Amendments from Version 1**

The updated article provides additional sources where necessary to further highlight the roles of galectin-3 in the innate immune system. Additionally, recent evidence is included that validates the S1-NTD of SARS-CoV2 as a promising therapeutic target. We hope this updated text to be a more detailed review with enhanced readability compared to the prior copy.

Any further responses from the reviewers can be found at the end of the article

Introduction

Galectin 3 (Gal-3) is a carbohydrate-binding protein 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). It is most highly expressed in myeloid cells (macrophages, dendritic cells, neutrophils, and monocytes), as well as epithelial cells, endothelial cells, and fibroblasts (Diaz-Alvarez & Ortega, 2017). When secreted by myeloid cells, Gal-3 and other galectins can act as modulators of cytokine expression by immune cells, and also as orchestrators of the damage associated molecular pattern (DAMP) system (Sato *et al.*, 2009). Recent discoveries specific to viral infections have begun to shed light on its role as well (Wang *et al.,* 2019). For example, in HIV and HTLV, Gal-3 serves as an attachment factor that facilitates viral entry into T-cells (Wang *et al.,* 2019). HIV infection also induces further Gal-3 expression through activation of NF-kB dependent pathways (Okamato *et al*., 2019). Secreted Gal-3 then mediates a number of deleterious effects. 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 (Caniglia *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-acetylneuraminic 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). Additionally, a neutralizing antibody against the SARS-CoV2 S1-NTD has been shown to completely inhibit cell entry by the virus (Chi *et al*., 2020). This indicates the NTD region is essential for viral entry and a promising therapeutic target (Chi *et al*., 2020). 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

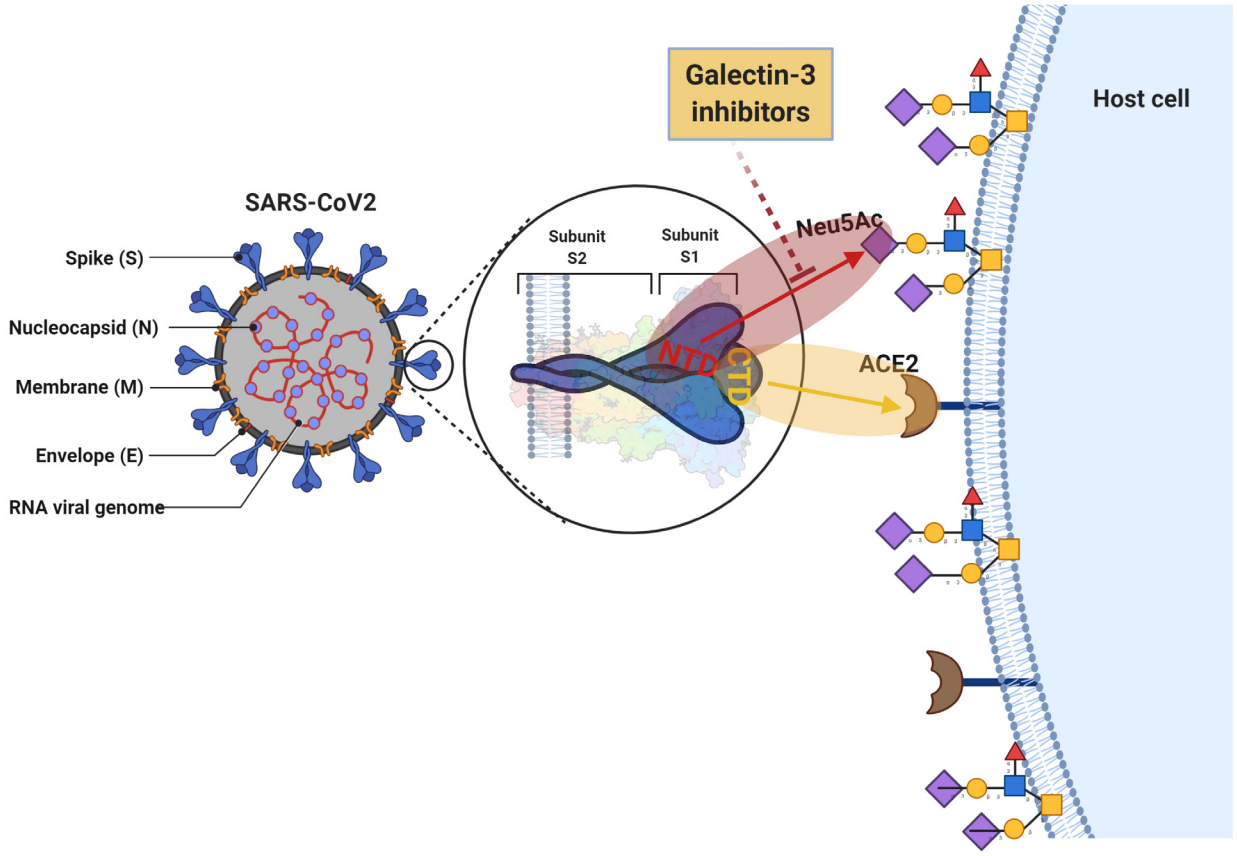

Figure 1. A dual attachment model for SARS-CoV2. Evidence has shown that a pocket in the NTD of SARS-CoV2 is capable of binding *N*-acetylneuraminic acid (Neu5Ac). This strongly supports a dual attachment model for SARS-CoV2, where NTD-Neu5Ac interactions facilitate initial host cell recognition by the virus and stabilize its entry via ACE2 receptors.

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 (Behloul *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 (Behloul *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 of Gal-3 and the S1-NTD of betacoronaviridae 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 (Caniglia *et al.*, 2020; 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 (Behloul *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 (Behloul *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). Similar findings were reported in a cohort of over 1,5000 patients, where serum IL-6 and TNF-α were found to be independent predictors of disease severity and mortality in COVID-19 (Del Valle *et al.*, 2020). This data speaks to the urgency of identifying therapeutics to reduce the incidence of CSS (Del Valle *et al.*, 2020; 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 (Chen *et al.*, 2015; Chen *et al.*, 2018; Ren *et al.*, 2019; Yip *et al.*, 2017). 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 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

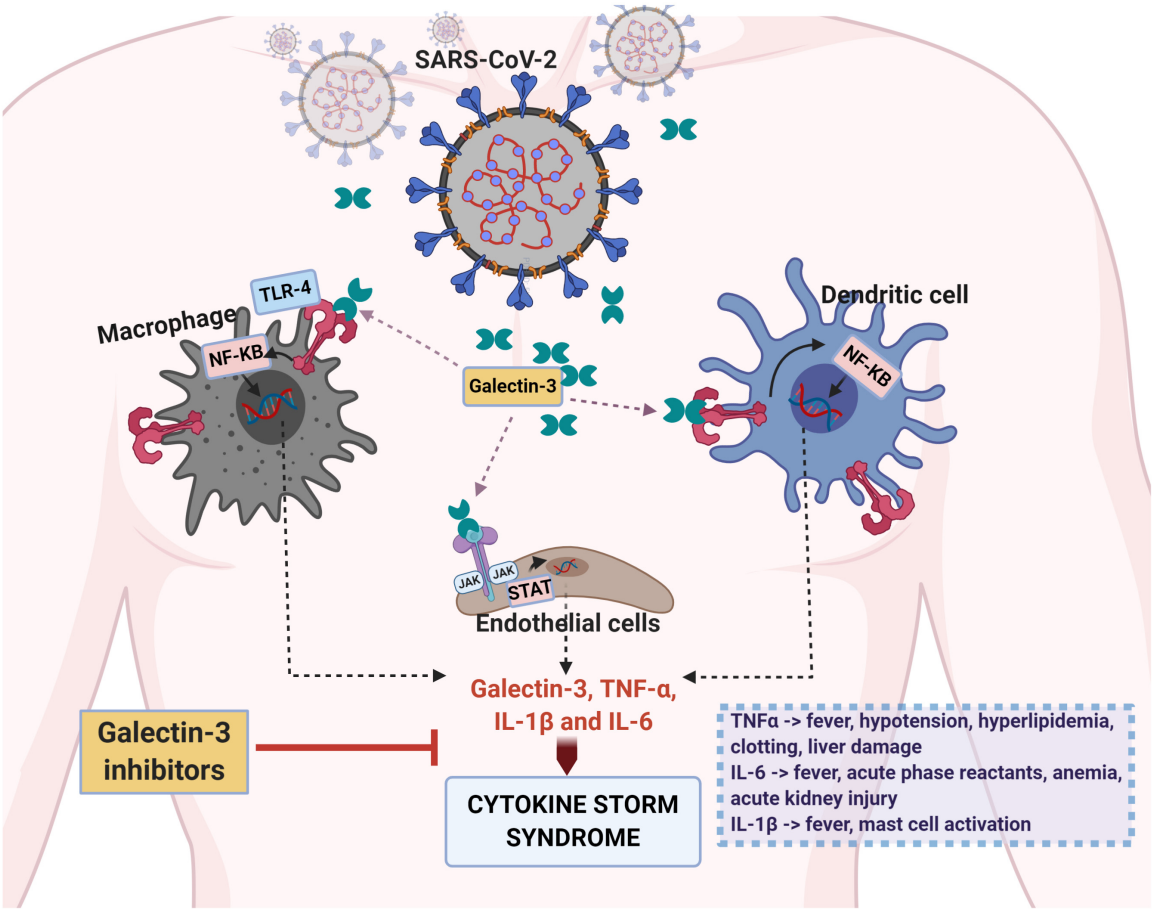

Figure 2. Gal-3 may amplify the cytokine storm syndrome associated with severe COVID-19. During severe SARS-CoV2 infection, increased plasma concentrations of Gal-3 are observed in circulating macrophages, monocytes, and dendritic cells. When secreted, Gal-3 can then agonize TLR4 receptors on their surfaces 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.

IL-1, IL-6, and TNF-α (Ren *et al.,* 2019; Yip *et al.,* 2017). Gal-3 K/O has also been shown to decrease both NF-kB activation and HIV viral replication in infected cells (Okamato *et al.*, 2019). 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). Additionally, in a cohort of recovered COVID-19 patients in Italy, 87.4% reported persistent symptoms, most notably fatigue and dyspnea, at an average of 60.3 days post-infection (Carfi *et al.*, 2020). This indicates that for some, persistent post-viral inflammation may result in deleterious changes such as pulmonary fibrosis (Crisan-Dabija *et al.*, 2020). Findings such as these 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 ß (TGF-ß), 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 go on to develop pulmonary fibrosis, and that these changes are mediated by a number of cytokines including TGF- ß, 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-ß 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 ß-catenin activation, indicating the beneficial effects were mediated via interruption of TGF-ß 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 treatment of COVID-19, the first clinical trial of a galectin inhibitor in COVID-19 to date (University of Edinburgh DEFINE trial, 2020).

Conclusions and future directions

In summary, Gal-3 is a lectin that exhibits a pleiotropic role in mediating the acute and chronic consequences of infection and inflammation. Multiple studies have shown Gal-3 to be highly upregulated in patients suffering from severe COVID-19 (De Biasi *et al.,* 2020; Kalfaoglu *et al.,* 2020; Liu *et al.,* 2020). On a cellular level, Gal-3 is most highly expressed in monocytes, macrophages, and dendritic cells during severe COVID-19 infection (Liu *et al.,* 2020). CSS complicated by the development of ARDS is the major cause of fatality in COVID-19 patients (Xu *et al.,* 2020b; Zhai *et al.,* 2020; Zhang *et al.,* 2020). This process is chiefly mediated by the release of IL-1, IL-6, and TNF-α from macrophages, monocytes, and dendritic cells (Zhang *et al.,* 2020). Gal-3 inhibition has been shown to reduce the release of these cytokines from immune cells (Chen *et al.,* 2015; Ren *et al.,* 2019; Yip *et al.,* 2017). Additionally, high Gal-3 is directly associated with worse outcomes and lower survival in ARDS patients (Xu *et al.,* 2017).

A key domain in the spike protein exhibits a high degree of morphological and sequence similarity to human Gal-3 (Behloul *et al.,* 2020). This NTD has been shown to bind Neu5Ac

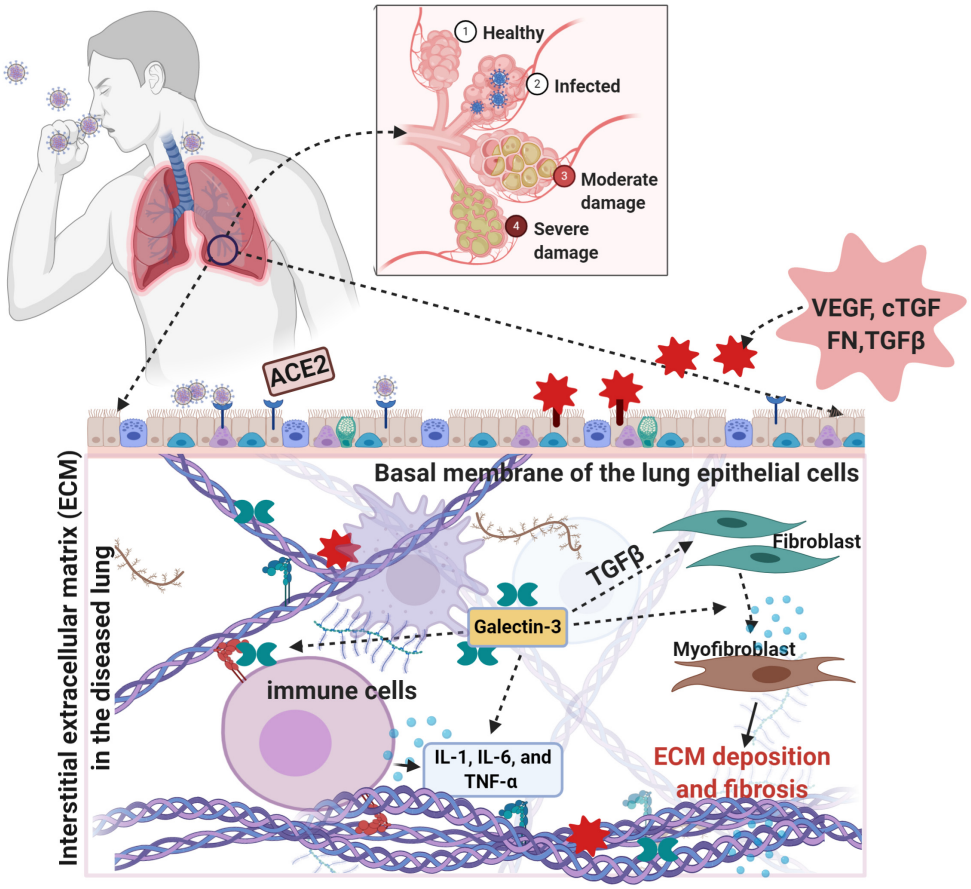

Figure 3. Gal-3 contributes to a pro-fibrotic microenvironment in COVID-19. During SARS-CoV2 infection, transcriptional upregulation of VEGF, TGF-ß, and fibronectin (FN) is seen in the pulmonary epithelium, creating a pro-fibrotic microenvironment. Secretion of Gal-3 by macrophages contributes to fibrosis by increasing the expression of TGF-ß receptors on the surface of fibroblasts. The fibroblasts and myofibroblasts are then activated by TGF-ß 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.

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-ß have been observed following SARS-CoV2 infection (Xu *et al.*, 2020a). Gal-3 secreted by macrophages during injury promotes the upregulation of TGF-ß 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 IIb 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.

Table 1. Search strategy for our literature review.

Database	Search Queries
PubMed	**On SARS-CoV2**: 'COVID-19 symptoms' 'SARS-CoV2 AND cytokine release syndrome' 'SARS-CoV2 entry mechanism' 'SARS-CoV2 AND galectins' 'SARS-CoV2 S1-NTD' 'SARS-CoV2 spike protein' 'SARS-CoV2 cytokine storm syndrome' 'SARS-CoV2 sialic acids' 'SARS-CoV2 fibrosis' **On β-coronaviruses**: 'MERS-CoV entry mechanism' **On Galectin-3**: 'Galectins' 'Galectin-3' 'Galectin-3 AND cytokines' 'Galectin-3 AND inflammation' 'Galectin-3 AND viruses' 'Galectin-3 AND viral infection' 'Galectin-3 AND fibrosis' **On Galectin-3 Inhibitors**: 'Galectin-3 inhibitors' 'TD139' 'TD139 pulmonary fibrosis'
Google Scholar	**On SARS-CoV2**: 'COVID-19,' 'COVID-19 symptoms' 'SARS-CoV2 fibrosis' **On Galectin-3:** 'Galectins' 'Galectin-3 cytokines' 'Galectin-3 fibrosis' **On Galectin-3 Inhibitors:** 'Galectin-3 inhibitors' 'TD139' 'Gal-3 clinical trials'

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.

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Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 05 October 2020

https://doi.org/10.5256/f1000research.29504.r72127

✔ **Talia H Swartz**
Division of Infectious Diseases, Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York City, NY, USA

I appreciate the authors' revisions and am happy to approve.

Competing Interests: No competing interests were disclosed.

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.

Version 1

Reviewer Report 16 September 2020

https://doi.org/10.5256/f1000research.28671.r70657

✔ **Thirunavukkarasu Velusamy**
Department of Biotechnology, Bharathiar University, Coimbatore, Tamil Nadu, India

- 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-3inhibitors 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 ofSARS-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?
Yes

Is the review written in accessible language?
Yes

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

Competing Interests: No competing interests were disclosed.

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.

Author Response 21 Sep 2020

Kiran Velpula, University of Illinois College of Medicine at Peoria, Peoria, USA

Response to Reviewer: Thirunavukkarasu Velusamy

- **The review article is clear, concise, and well structured.**
  - We appreciate the above comment. Thank you.

- **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-3inhibitors 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.**

- T**he 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 ofSARS-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
  - The reviewer brings up a great point. To address this question, we have added a citation that shows the detection of a neutralizing antibody against the NTD of SARS-CoV2 S1 protein. This antibody is effective at completely neutralizing SARS-CoV2 cell entry even in the absence of other antibodies that bind the receptor binding domain or ACE2 receptors. With this in mind, it follows that a drug such as a galectin inhibitor targeting the NTD may be effective as a standalone therapy. The reviewer brings up a good point regarding combination therapy, in that combination of NTD / CTD targeted therapies may be a better long term treatment strategy given the mutagenicity of the virus' spike protein.

- **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.**
  - Thank you for providing this citation. We are certainly glad to see other laboratories participating in this field of research. However, given that this article is also a review article and not primary research, we do not see a role for it to be cited in our current review article, which aims to summarize primary research findings regarding galectin-3 and COVID-19.

- Overall the article is well-written and recommended for publication once the minor corrections have been addressed.

Competing Interests: We do not have any competing interests.

Reviewer Report 08 September 2020

https://doi.org/10.5256/f1000research.28671.r70959

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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 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7301894/[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

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2. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, et al.: An inflammatory cytokine signature predicts COVID-19 severity and survival.*Nat Med*. 2020. PubMed Abstract | Publisher Full Text

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.

Author Response 08 Sep 2020

Kiran Velpula, University of Illinois College of Medicine at Peoria, Peoria, USA

Response to Reviewer: Talia Swartz, MD, PhD

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 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7301894/[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 reviewer makes a great point. With the title of this work we are trying to convey a distinction in so far as the prior article explicitly argued for the repurposing of a drug toward COVID-19 treatment. In this article, we are discussing the pathologic effects Gal-3 may exert in severe COVID-19, hence the title.
- **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).**
  - Thank you for providing the additional references. We have added a couple sentences to more explicitly detail the roles of Gal-3 in viral infection and have incorporated the findings of Okamato et. al into multiple sections of the review.
- **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.**
  - The reviewer makes an excellent point. We have added additional sources (Diaz Alvarez et. al 2017; Sato et. al 2009) to provide further introduction to the role of galectins in infections and to reference the cell types Gal-3 is most highly expressed in.
- **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.**
  - We have revised the sentence to more explicitly define the structural similarities of Gal-3 and S1-NTD of coronavirus spike proteins. We have also added an additional reference of Caniglia et. al.
- **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]

- Thank you for providing the additional citation. We will certainly add these findings to the manuscript.

- **Figure 1 does not add richly to this work and perhaps could be a panel combined with Figure 2.**
  - Thank you for this comment. We believe Figure 1 to be essential as it details the likely role of the galectin-like S1-NTD in COVID-19 infection. To further highlight the importance of this figure and the NTD, we have added a recent publication (Chi et. al, 2020) which shows a neutralizing antibody against the NTD inhibits viral entry. It then follows that if a drug such as a galectin inhibitor is able to also bind this region of the NTD, it may also inhibit cell entry by SARS-CoV2.
- **The authors state "Several studies to date have shown the effects of anti-Gal-3 therapy on cytokine release." These studied should be cited.**
  - We have added the appropriate sources to support this statement.
- **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 reviewer makes an excellent point. We have updated the figure legend to show that Gal-3 is produced in circulating immune cells in the 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.**
  - The reviewer makes an excellent point here. We have added multiple sources to this section that we believe better characterize the chronic inflammatory signature some COVID-19 patients report months after the initial infection. We have also included an additional citation of an excellent commentary on the concern for development of post-infectious IPF in COVID-19 patients.

Competing Interests: No competing interests were disclosed.