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Full Length Article

SARS-CoV-2 proteins regulate inflammatory, thrombotic and diabetic responses in human arterial fibroblasts

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ABSTRACT

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for many pathological processes, including altered vascular disease development, dysfunctional thrombosis and a heightened inflammatory response. However, there is limited work to determine the underlying cellular responses induced by exposure to SARS-CoV-2 structural proteins. Thus, our objective was to investigate how human arterial adventitial fibroblasts inflammation, thrombosis and diabetic disease markers are altered in response to Spike, Nucleocapsid and Membrane-Envelope proteins. We hypothesized that after a short-term exposure to SARS-CoV-2 proteins, adventitial fibroblasts would have a higher expression of inflammatory, thrombotic and diabetic proteins, which would support a mechanism for altered vascular disease progression. After incubation, the expression of gC1qR, ICAM-1, tissue factor, RAGE and GLUT-4 was significantly up-regulated. In general, the extent of expression was different for each SARS-CoV-2 protein, suggesting that SARS-CoV-2 proteins interact with cells through different mechanisms. Thus, SARS-CoV-2 protein interaction with vascular cells may regulate vascular disease responses.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that has been shown to target many different organ systems, leading to inappropriate immune and thrombotic reactions [31] [46]. These altered reactions lead to significant pathologies that are exacerbated in patients with pre-existing and/or underlying medical conditions. Many reports illustrate that patients with vascular pathologies who become infected with SARS-CoV-2 have higher mortality and morbidity rates as compared to patients without vascular pathologies [15] [47]. The cellular and molecular responses and mechanisms that link vascular pathologies, such as diabetes, SARS-CoV-2, and enhanced mortality/morbidity rates are currently unknown. As vascular diseases are also characterized by enhanced inflammatory and thrombotic reactions [4] [48], it is important to evaluate the interactions that can link these functions during SARS-CoV-2 exposures.

Various proteins are expressed by SARS-CoV-2 and each are thought to be responsible for viral interactions with human cell types: namely, Spike (S) Protein, Nucleocapsid (N) Protein and Membrane-Envelope (M) Protein [24]. It has been observed that S-protein can interact with the ACE2 receptor [38], N-protein can interact with NF-κB signaling and interferon production [17] and M-protein inhibits inflammasome activation [52]. However, proteins from other viral sources have been shown to interact with gC1qR (the receptor for the globular head of C1q), instigating altered inflammatory responses [6] [11] [42]. Further, due to the role of gC1qR in vascular inflammation, thrombosis and hemostasis, and gC1qR's expression on multiple cell types that are important for vascular disease development, we investigated the expression of gC1qR in response to SARS-CoV-2.

Vascular complications, including altered inflammatory and thrombotic response, are hallmarks of vascular pathologies and have been correlated with altered mortality and morbidity rates in patients [2] [41]. Under these conditions, it is common to observe enhanced innate immune reactions and thrombotic reactions, possibly due to the altered signaling of gC1qR, which can link inflammation and thrombosis within the vascular system. Specifically, gC1qR activates and induces intracellular signaling mechanisms in response to C1q binding and high molecular weight kininogen (HK) binding [12]. C1q has a direct role in the complement cascade aiding in the capture, docking and cleavage of C4 to C4b [3]. Enhanced actions of HK play a role in both bradykinin activity and the activation of Factor XII, C3, and C5, thus illustrating a convergence of inflammation and thrombosis as a function of gC1qR

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activity [5] [14]. Downstream of these activities, it is common to observe heightened ICAM-1 and tissue factor expression, which have become markers of vascular complications.

Diabetic conditions have been shown to enhance both vascular inflammation and thrombosis [19] [50]. Additionally, diabetic patients who are exposed to SARS-CoV-2 and present with COVID-19 have been shown to have significantly enhanced mortality and morbidity rates [18]. Unfortunately, the molecular interactions of these two conditions have not been identified, although there is emerging evidence that advanced glycation end products (AGEs) and the signaling of these molecules alters disease progression, inflammation and thrombosis [35] [36], primarily through the interaction of AGEs and the receptor for AGEs (or RAGE) [30]. Further, diabetic patients are characterized by a disturbance in glucose uptake through glucose transporters (GLUTs), which may be caused by a deficiency in GLUT translocation to the cell membrane via altered insulin receptor signaling [27]. Thus, the combination of altered glucose uptake (from altered GLUT expression) and enhanced thrombosis and inflammation (from altered RAGE signaling) are thought to be two of the primary causes of diabetic induced vascular pathologies.

Here we chose to investigate the effects of SARS-CoV-2 S-, N- and M-proteins on human arterial adventitial fibroblasts immune, thrombotic and diabetic responses that have previously been correlated to cardiovascular disease progression and that may be responsible for enhanced mortality and morbidity rates in COVID-19 positive patients with underlying vascular complications. We hypothesized that SARS-CoV-2 proteins would interact with fibroblast gC1qR to initiate significantly enhanced inflammatory, thrombotic and diabetic reactions as compared with fibroblasts that were not exposed to viral proteins. Additionally, these altered responses may serve as markers for COVID-19 disease progression in patients with underlying vascular pathologies.

2. Methods and materials

2.1. Cells

Human aortic adventitial fibroblasts (AFs) were purchased from ScienCell Research Laboratories (Carlsbad, CA) and maintained in fibroblast medium-2 supplemented with 5% fetal bovine serum, antibiotics (penicillin/streptomycin) and growth supplement (as suggested by the manufacturer) at 37 °C and 5% CO_2. AFs were cultured on tissue culture plastic flasks and well-plates. At confluence, cells were passaged with trypsin digestion for approximately 3 min. For experiments, AFs were incubated with SARS-CoV-2 S-protein, N-Protein or M-protein (Virogen, Watertown, MA) at a final concentration of 3 μg/mL for 1 h. All experiments included an internal negative control consisting of AFs exposed to media only for the entire duration. For statistical purposes, the seeding density of cells for all experiments was maintained at approximately 1000 cells/cm^2.

2.2. AF viability, density and metabolic activity

In order to determine whether or not the incubation of AFs with SARS-CoV-2 proteins induced changes in cell culture parameters, we made use of a standard live/dead cell cytotoxicity assay and the MTT assay to quantify AF viability, density and metabolic activity after the exposure conditions. Briefly, the live/dead cell cytotoxicity assay consisted of 2 μM calcein and 4 μM ethidium (ThermoFisher Scientific). After the exposure conditions, AFs were washed with warmed PBS (pH 7.4, 37 °C) and immediately incubated with ~50-100 μL of the calcein/ethidium mixture for approximately 3–5 min. After the incubation, cells were immediately imaged on an inverted microscope as previously described [34] [35]. 3 random locations were imaged per independent tissue culture well. The data from each independent well was averaged for a single data point. Cell viability is defined as the number of live cells in the imaging area divided by the total number of cells per imaging area [32]. Cell density is defined as the total number of live cells per imaging area, which has been calibrated for each of our microscope objectives. Cell density is then normalized by the initial cell seeding density to provide us with a measure of proliferation over the time interval [37].

To quantify AF metabolic activity after the exposure conditions, we made use of a 3- [4,5-dimethylthiazol-2-yl] − 2,5-diphenyl tetrazolium bromide (MTT) assay [23] [33], which quantifies the activity of mitochondrial dehydrogenase (note that all MTT reagents were purchased from Millipore Sigma, St. Louis, MO). AFs were washed with warm PBS (pH 7.4, 37 °C) and then immediately incubated with MTT reagent reconstituted in basal AF media for approximately 2 h. Formazan crystals were dissolved in 10% Triton-X and 0.1 M HCl in anhydrous isopropanol. The ensuing solution was gently mixed on an orbital shaker for 10–15 min. The absorbance of the solution resulting from the dissolving procedures was quantified at 690 nm. Duplicate 100 μL samples were collected from each independent condition and transferred to a 96 well plate for absorbance measurements and to ensure data accuracy. All data was normalized to the metabolic activity of paired cells incubated in the absence of SARS-CoV-2 structural proteins.

2.3. gC1qR expression

AF gC1qR expression was quantified after the exposure conditions using a solid-phase ELISA approach. Cells were washed (2×, PBS, pH 7.4), fixed with 0.5% glutaraldehyde (15 min, 37 °C, pH 7.4) and then neutralized with 100 mM glycine – 0.1% BSA (30 min, 37 °C). Protein expression was assessed with anti-gC1qR monoclonal antibodies (60.11, 74.5.2 and 83.13 epitopes) in independent wells. These protein regions are associated with different structural and functional domains of gC1qR, as previously described [7] [28], with the addition that the 83.13 epitope is associated with the HK binding site on gC1qR. All primary antibodies were incubated with AFs for 1 h at a final concentration of 1 μg/mL. To detect primary antibody binding, cells were washed and then incubated with appropriate alkaline phosphatase conjugated secondary antibodies (Millipore Sigma) at a final concentration of 1 μg/mL for 1 h. Color development was achieved by addition of pNPP and the absorbance was read spectrophotometrically at 405 nm. Note that all details have been reported by us previously and that all appropriate negative and positive controls were included within each independent experiment [34] [51].

2.4. Inflammatory, thrombotic and diabetic marker expression

The surface expression of AF ICAM-1, tissue factor, RAGE and GLUT-4 was quantified after the exposure to SARS-CoV-2 proteins using a solid-phase ELISA approach. Similar to the methods described for gC1qR expression, after the exposure conditions, AFs were washed, fixed and neutralized and then incubated with specific monoclonal antibodies towards the proteins of interest for one hour (RAGE and GLUT-4 primary antibodies were purchased from Abcam (Cambridge, MA); ICAM-1 and tissue factor primary antibodies were purchased from Invitrogen (Carlsbad, CA)). Primary antibody concentrations were as follows: ICAM-1, 1 μg/mL; tissue factor, 5 μg/mL; RAGE, 1 μg/mL; GLUT-4, 1 μg/mL. To detect primary antibody binding, cells were washed and then incubated with appropriate alkaline phosphatase conjugated secondary antibodies (Millipore Sigma) at a final concentration of 1 μg/mL for 1 h. As above, color development was achieved by addition of pNPP and the absorbance was read spectrophotometrically at 405 nm.

2.5. Statistics

All viability, density and metabolic activity data was normalized as described above. All ELISA data from each independent experiment was normalized to the AF control samples (e.g. cells incubated for the same duration in the absence of SARS-CoV-2 proteins), with background subtraction, as appropriate. Note that since there were no statistical

differences in the observed metabolic activity of cells after 1 h of exposure to SARS-CoV-2 proteins, we did not normalize ELISA data by cell growth characteristics. When there were multiple dependent wells, this data was first averaged to obtain a single independent data point for the particular condition. Normalized data from at least 4 independent experiments are shown and used for statistical analysis (all sample size numbers are reported in the Figure legends). Statistical analysis was carried out in SAS (v 9.4, SAS Institute, Cary, NC) using a one-way ANOVA procedure (factor is the exposure condition) and the Duncan post-host test. Note that all exposures were compared to a 'Negative Control,' which was cells incubated for the appropriate duration in standard cell culture media.

3. Results

3.1. AF viability, density and metabolic activity

In order to determine the effects of SARS-CoV-2 structural proteins on adventitial fibroblast culture conditions, we quantified cell viability, density and metabolic activity (Fig. 1). Cell viability is a measure of whether or not the exposure conditions directly cause cell death. After 24 h of exposure to SARS-CoV-2 proteins, we observed no changes in cell viability (Fig. 1A). Cell density is a measure of whether or not the exposure conditions can impair cell growth. After 24 h of exposure to each SARS-CoV-2 proteins, we observed a significant reduction in cell density as compared with our negative control (ANOVA, $P < 0.05$, Fig. 1B). For all conditions, the metabolic activity (Fig. 1C) was relatively high and not a function of incubation with SARS-CoV-2 proteins as compared with our negative control. Thus, the exposure of adventitial fibroblasts to SARS-CoV-2 proteins does not directly inhibit cellular metabolism or viability, but does impair cell growth over 24 h. All ELISA data was quantified after 1 h of exposure, similar to our metabolic activity quantification. Since there was no impairment in metabolic activity, no further normalization of ELISA data was conducted.

3.2. AF gC1qR expression

In order to determine whether or not SARS-CoV-2 exposure alters the expression of specific gC1qR activity sites, we used three monoclonal antibodies that target different structural and functional units of the gC1qR protein to quantify the expression of gC1qR (Fig. 2). The globular head of C1q binds to gC1qR at a site that is recognized by the 60.11 monoclonal antibody (Fig. 2A). We observed a significant decrease in the expression of gC1qR as quantified by the 60.11 antibody in response the exposure to each of the three SARS-CoV-2 proteins as compared with the negative control ($P < 0.05$, ANOVA). Exposure to M-protein did not inhibit the expression of gC1qR as quantified by 60.11 as significantly as the exposure to S-protein and the N-protein ($P < 0.05$, ANOVA); however, there was still an approximate 50% reduction in 60.11 antibody binding after exposure to M-protein as compared with the negative control.

gC1qR expression was also assessed with a monoclonal antibody that targets the domain of gC1qR that is associated with the activation of the kinin and coagulation systems (74.5.2, Fig. 2B). We also observed a significant decrease in the antibody binding to this protein domain after adventitial fibroblasts were exposed to each of the three SARS-CoV-2 proteins ($P < 0.05$, ANOVA) as compared with fibroblasts in the absence of SARS-CoV-2 proteins. Similar to the C1q binding site measurements, the exposure to N-protein did not inhibit the expression of gC1qR as quantified by 74.5.2 as significantly as M-protein and S-protein ($P < 0.05$, ANOVA), however there was still an approximate 25% reduction in response to N-protein exposure. We also assessed the HK binding domain of gC1qR with the 83.13 monoclonal antibody (Fig. 2C). After exposure to each of the SARS-CoV-2 proteins, there was a significant increase (approximately 10%) in the binding of the 83.13 antibody as compared with cells that were not exposed to any SARS-CoV-2 protein

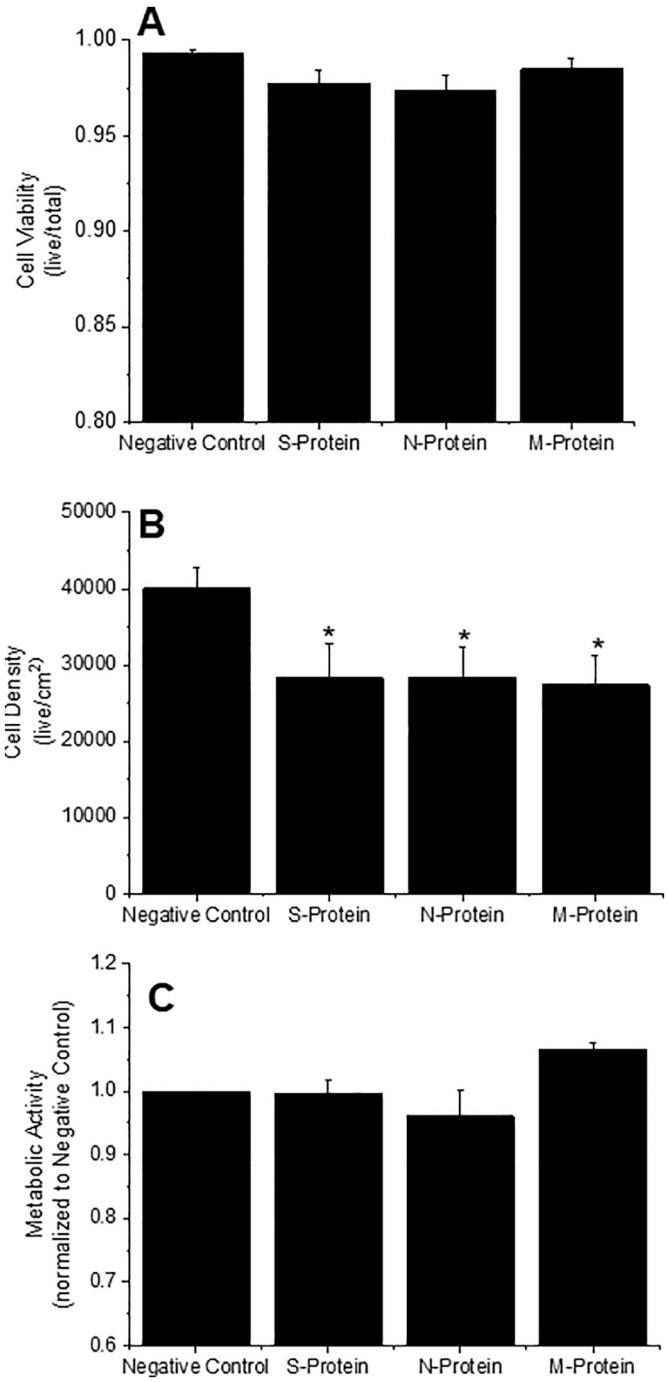

Fig. 1. Adventitial fibroblast viability (A), density (B) and metabolic activity (C) after the exposure to SARS-CoV-2 Spike (S) Protein, Nucleocapsid (N) Protein and Membrane-Envelope (M) Protein for 24 h (A and B) or 1 h (C). All data are reported as the mean + standard error of the mean for 9 independent experiments (for viability/density) and 3 independent experiments (for metabolic activity). *Significantly different than negative control (ANOVA, Duncan method).

($P < 0.05$, ANOVA) and there were no significant differences between the SARS-CoV-2 proteins.

3.3. AF gC1qR inflammatory, thrombotic and diabetic markers

We also aimed to determine whether or not the exposure to SARS-CoV-2 structural proteins could alter the inflammatory and thrombotic potential of adventitial fibroblasts. Further, we investigated whether or

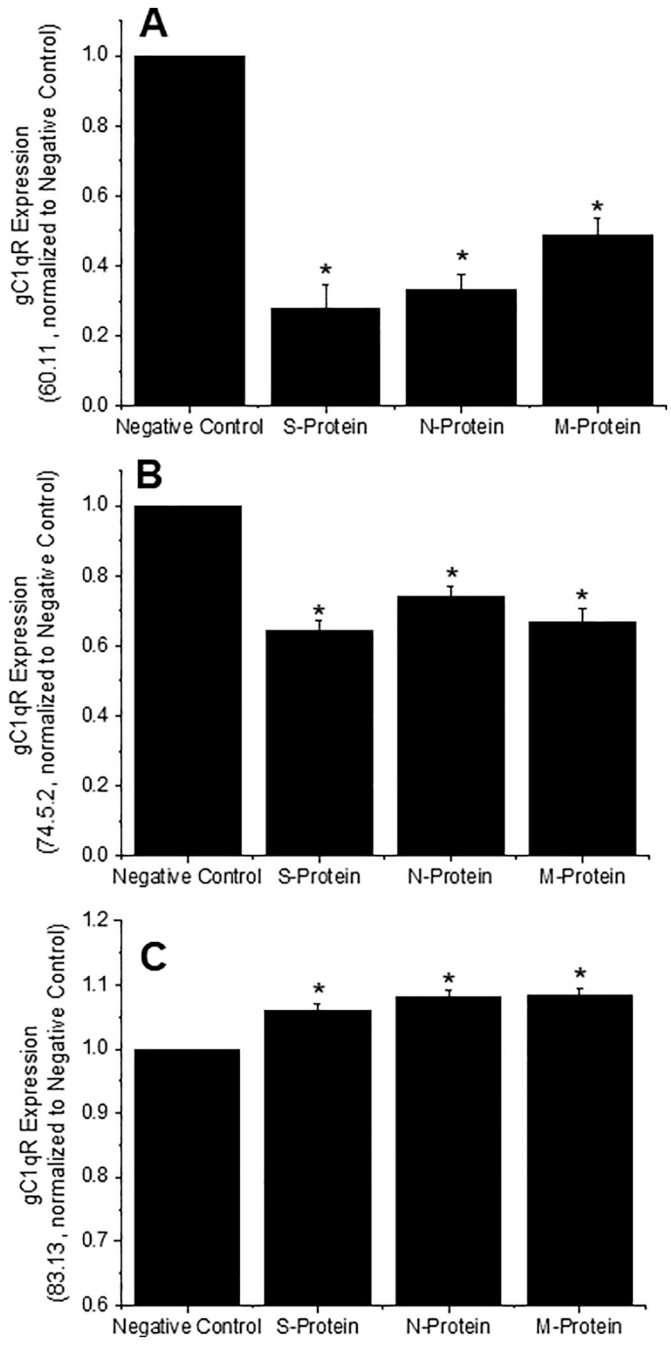

Fig. 2. gC1qR expression on the cell membrane of adventitial fibroblasts after exposure to SARS-CoV-2 Spike (S) Protein, Nucleocapsid (N) Protein and Membrane-Envelope (M) Protein for 1 h. We observed different protein locations that are associated with different structural and functional locations of gC1qR: including 60.11 (A, the C1q globular head binding domain), 74.5.2 (B, activation of kinin and coagulation systems) and 83.13 (C, the HK binding domain). All data are reported as the mean + standard error of the mean for a minimum of 4 independent experiments (range 4–14). *Significantly different than negative control (ANOVA, Duncan method).

not SARS-CoV-2 proteins could alter the expression of proteins associated with diabetes progression. ICAM-1 expression was significantly enhanced for cell populations that were exposed to N-protein or M-protein but not S-protein as compared with cells that were not exposed to SARS-CoV-2 proteins ($P < 0.05$, ANOVA, Fig. 3A). Further, we also observed a significant difference in ICAM-1 expression for cell populations exposed to N-protein and M-protein as compared with cells exposed to S-protein ($P < 0.05$, ANOVA). Tissue factor expression was also quantified after exposure to SARS-CoV-2 proteins. S-protein and N-protein induced a significantly enhanced expression of tissue factor as compared with cells that were not exposed to any SARS-CoV-2 proteins ($P < 0.05$, ANOVA, Fig. 3B), and there were no statistical differences between tissue factor expression after exposure to any of SARS-CoV-2 proteins (compared amongst the proteins).

As diabetic patients who present with COVID-19 have higher mortality and morbidity rates, we investigated the expression of RAGE and GLUT-4 after exposure to SARS-CoV-2 proteins (Fig. 3C and D, respectively). Exposure to S-protein significantly enhanced the expression of RAGE ($P < 0.05$, ANOVA) as compared with cells not exposed to SARS-CoV-2 proteins; whereas the expression of RAGE was independent of the presence of N-protein or M-protein. GLUT-4 expression was significantly enhanced after exposure to N-protein ($P < 0.05$, ANOVA) as compared with cells not exposed to viral proteins (Fig. 3D). Further, there were no statistical differences in GLUT-4 expression between the exposures to the different SARS-CoV-2 proteins.

4. Discussion

4.1. AF viability, density and metabolic activity

In order to determine whether or not exposure to SARS-CoV-2 proteins can impair cell growth, we quantified cell viability and density (Fig. 1A and B, respectively) after 24 h of exposure. Note that we did not quantify this data after 1 h of exposure as this would not be sufficient time to elicit quantifiable changes in viability or density. Our data suggests that there is no direct effect of SARS-CoV-2 proteins on cell death (e.g. cell viability was high for all SARS-CoV-2 proteins), however, there was an impairment in cell growth (e.g. cell density was significantly reduced for all SARS-CoV-2 proteins). As viruses require the host cells to survive to produce new viruses, this data would be in agreement with the typical mechanisms that a virus employs. Other groups have shown that under certain conditions viruses can cause reductions in cell viability, but in general reduced cell viability after exposure to viruses is not observed [13] [43]. Cell density is a measure of cell proliferation and apoptosis as a function of time. There are conflicting reports in the literature regarding the effects of viral infection on fibroblast apoptosis; where some groups report an inhibition of apoptosis [54] and others report an inducement of apoptosis [53]. While the role of viral infection on apoptosis may be a function of the viral association and viral protein entry mechanisms, others have shown that SARS-CoV-1 can increase DNA fragmentation and apoptosis via a TUNEL assay [39], which is in agreement with our data. Metabolic activity of adventitial fibroblasts was examined to determine whether or not the exposure to SARS-CoV-2 proteins alters the metabolism of cells. Our data suggests that the exposure to SARS-CoV-2 proteins does not alter adventitial fibroblasts metabolic activity (Fig. 1C). Although there are no other reports to directly compare to, the cytotoxicity of various viruses to fibroblasts cell lines have been investigated. In general, there are small but non-significant reductions when fibroblasts are exposed to infectious bursal disease virus [45] and adenovirus-tumor suppressor [20], however, HCV has been shown to lead to cell dysfunction that would eventually lead to apoptosis [29]. Thus, our data on the effects of SARS-CoV-2 viral proteins on the metabolic activity of fibroblasts is in agreement with previous work and suggests that cells that remain after the initial exposure can continue to function in a non-impaired manner and maintain normal mitochondrial activity.

4.2. AF gC1qR expression

The expression of adventitial fibroblast gC1qR, as measured by three monoclonal antibodies that target different functional and structural domains, was assessed to determine (1) the ability of SARS-CoV-2 to interact with the innate immune system and (2) the ability for SARS-

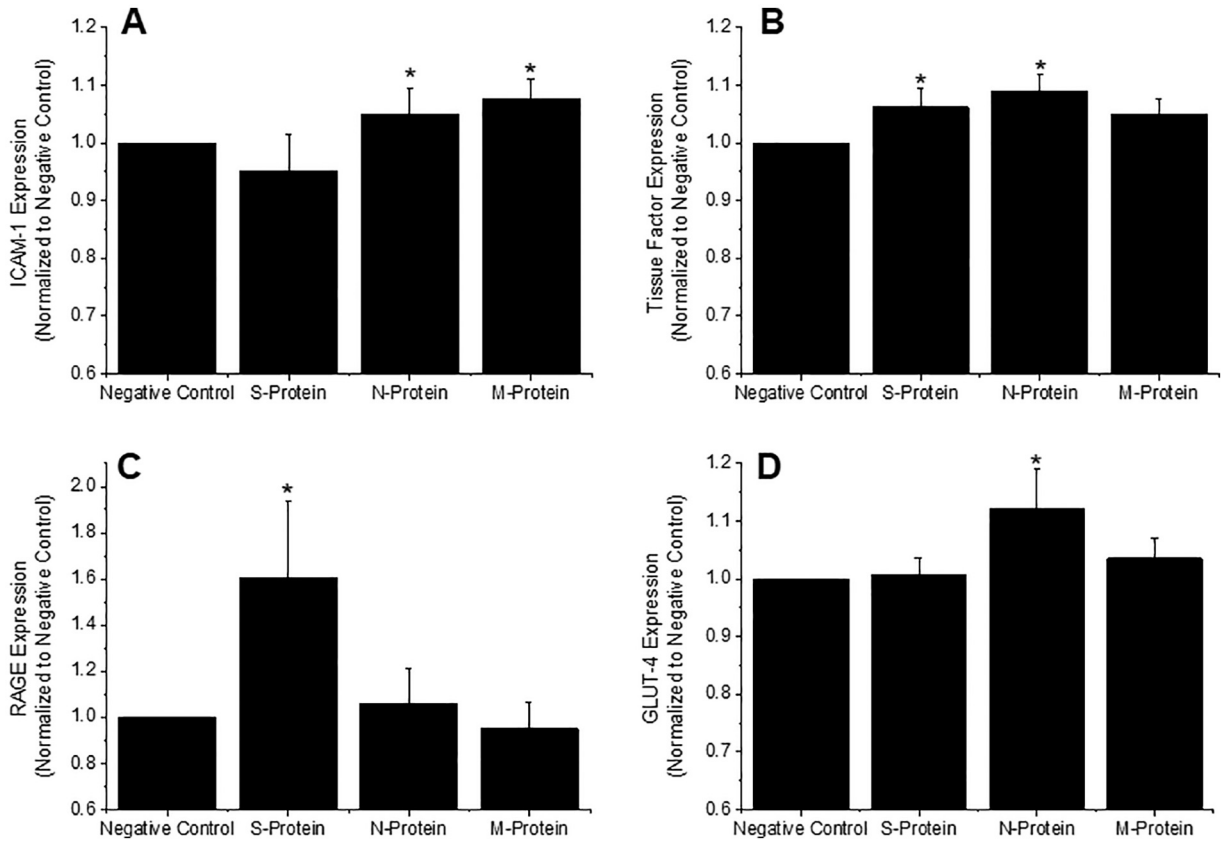

Fig. 3. ICAM-1 (A), Tissue Factor (B), RAGE (C) and GLUT-4 (D) expression on the cell membrane of adventitial fibroblasts after exposure to SARS-CoV-2 Spike (S) Protein, Nucleocapsid (N) Protein and Membrane-Envelope (M) Protein for 1 h. All data are reported as the mean + standard error of the mean for a minimum of 4 independent experiments (range 4–5 for ICAM-1, 7 for tissue factor, 6–7 for RAGE and 6–7 for GLUT-4). *Significantly different than negative control (ANOVA, Duncan method).

CoV-2 proteins to bind to gC1qR at different functional locations. Our data shows that after exposure to SARS-CoV-2 proteins the expression of gC1qR as assessed by the 60.11 and 74.5.2 antibodies was significantly inhibited (Fig. 2A and 2B). 60.11 binding is associated with the C1q binding domain and 74.5.2 is associated with the kinin and coagulation system activation domains. Thus, our data would suggest that each of the SARS-CoV-2 proteins can interact and associate with these gC1qR domains, reducing the likelihood for primary antibody binding. This is in agreement with previous work that shows that viral infections can bind to and antagonize responses from gC1qR, including the porcine circovirus [6], adenovirus core protein [25], HIV [22] and HCV [16]. Thus, it is probably that SARS-CoV-2 is interacting with host cells through interactions between viral structural proteins and gC1qR. Further, these interactions have the potential to antagonize innate inflammatory pathways that are commonly observed during physiological and pathological responses.

In contrast to gC1qR expression as detected by the 60.11 and 74.5.2 monoclonal antibodies, the expression of gC1qR as quantified by the 83.13 antibody, was significantly increased after exposure to each of the SARS-CoV-2 proteins (Fig. 2C). The 83.13 clone recognizes the HK binding site and is associated with the activation of kinin pathways. It is thought that the gC1qR HK binding domain is one of the primary links between the innate immunity and contact activation of the coagulation system [10] and a regulator of bradykinin signaling [9] (through BR-1) and production [8]. Although there has been limited work on how viral infections alter the expression of the gC1qR HK binding site, our data suggests that there is an up-regulation of either gC1qR directly, which is not observed with the 60.11 and 74.5.2 monoclonal antibodies due to the possible interaction of viral proteins with the receptor, or that the HK binding site becomes more available after viral infection, without a change in protein expression. Further, our data suggests a link between viral infection, innate immune reactions and thrombotic reactions which has been observed in COVID-19 positive patients, who have higher mortality and morbidity rates with underlying vascular complications [21].

4.3. AF inflammatory, thrombotic and diabetic markers

To correlate our observations of viral interactions with gC1qR, we monitored the expression of common inflammatory, thrombotic and diabetic markers in adventitial fibroblasts after exposure to SARS-CoV-2 proteins. ICAM-1 expression (Fig. 3A) is commonly observed during inflammatory pathologies and is associated with cytokine production/signaling and viral entry. Our data suggests that there is an increase in ICAM-1 in response to the exposure to N-protein and M-protein, but not S-protein. This is in agreement with findings that show ICAM-1 can be upregulated in fibroblasts after exposure to orf virus [40] and HCV [29]. Similarly, tissue factor expression was up-regulated after exposure to S-protein and N-protein, but not M-protein (Fig. 3B). Tissue factor is associated with the initiation of extrinsic coagulation and many vascular pathologies including atherosclerosis. Viral infections have been previously associated with altered coagulopathy, including the hemorrhagic fever virus induced enhanced fibrin deposition on fibroblastic reticular cells [44] and the respiratory viruses (including influenza and cytomegalovirus) that increase tissue factor expression in endothelial cells, the generation of Factor Xa and assembly of the prothrombinase complex [49]. Our data confirms these findings and shows that SARS-CoV-2 can interact with adventitial fibroblasts to increase thrombotic activity through tissue factor expression.

Diabetic patients who present with COVID-19 have been shown to

have enhanced mortality and morbidity rates as compared with COVID-19 patients without diabetes. Thus, we aimed to investigate if SARS-CoV-2 proteins can modulate the expression of common proteins observed during diabetes progression (Fig. 3C and D). Our data shows that exposure to S-protein induces an enhanced expression of RAGE and the exposure to N-protein induces an enhanced expression of GLUT-4 on adventitial fibroblasts. RAGE gene expression has been shown to be enhanced after SARS-CoV-2 exposure [1] and our data confirms that this can be observed at the protein level as well; however, in response to S-protein only. Human adenovirus (Ad36) has been observed to increase GLUT-4 expression in adipocytes and this was correlated to inflammatory changes observed during the specific exposure conditions [26]. Combining our work with previous findings suggests that viral infections can interact with and exacerbate the progression of diabetes. Additionally, since SARS-CoV-2 can alter inflammatory and thrombotic pathways, it is possible that all of these reactions combine to lead to an enhanced inflammatory and thrombotic state for patients that present with COVID-19 and underlying vascular complications.

5. Conclusions

SARS-CoV-2 has resulted in an unprecedented global pandemic. Emerging clinical data suggests that patients who present with COVID-19 have dysregulated inflammatory and thrombotic responses that are perpetuated when there are underlying medical conditions, such as diabetes. In this report, we show that exposure to SARS-CoV-2 structural proteins can alter the expression of distinct gC1qR domains that are associated with different functional responses. As gC1qR has emerged as an important link between inflammation and thrombosis, we investigated adventitial fibroblast ICAM-1 and tissue factor expression. We observed an enhanced expression of these markers in response to particular SARS-CoV-2 proteins. Additionally, we show that SARS-CoV-2 can interact with and potentially alter the progression of diabetes by modulating RAGE expression and GLUT-4 expression. Thus, our data supports the observations that SARS-CoV-2 can alter pathological responses that may associate with the heightened mortality and morbidity rates in patients with underlying medical conditions. Furthermore, our data supports the role of gC1qR as a docking protein for viral infection and a mechanism to alter inflammatory and thrombotic processes.

Combining our observations, we have developed a working hypothesis for how SARS-CoV-2 structural proteins interact with adventitial fibroblasts to increase the likelihood for vascular disease instigation, activation and/or progression (Fig. 4). Data from our group has shown that SARS-CoV-2 structural proteins can interact with gC1qR, expressed on various cell types, including endothelial cells (manuscript under review). Thus, it is our belief that endothelial gC1qR can serve as a docking receptor for SARS-CoV-2. We have also shown that the interaction of gC1qR and SARS-CoV-2 structural proteins increases the production of high molecular weight kininogen and bradykinin (manuscript under review), which increases vascular permeability. Under conditions when SARS-CoV-2 has access to the sub-endothelial space (e.g. during vascular diseases, vascular wall damage, or increased vascular permeability), it is possible for SARS-CoV-2 structural proteins to interact with sub-endothelial gC1qR. Here we show that the interaction of SARS-CoV-2 structural proteins with adventitial fibroblasts, increases the expression and/or activation of gC1qR (positive feedback loop to potentially increase inflammatory, thrombotic and edema responses), tissue factor (increases the likelihood for extrinsic coagulation activation in the presence of factors VIIa and X), ICAM-1 (increases the likelihood of vascular inflammation under disease conditions), RAGE (increases chronic inflammation and thrombosis under diabetic conditions), and GLUT-4 (induces hypoglycemic conditions). Under vascular disease

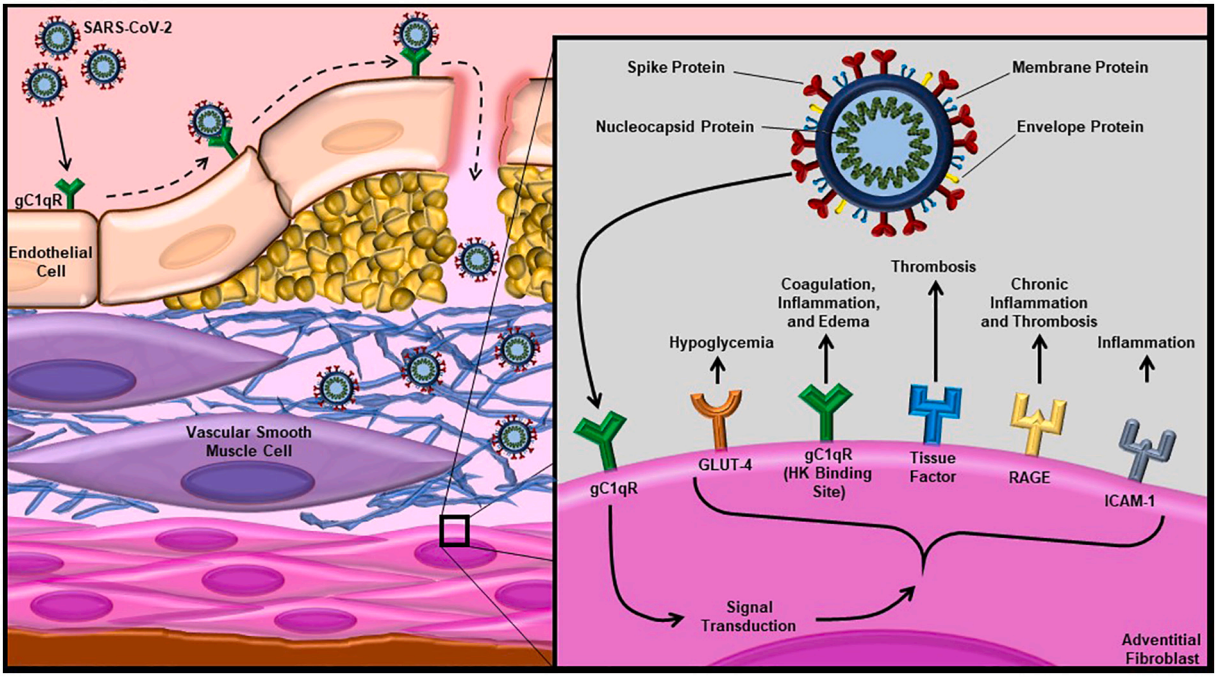

Fig. 4. Proposed model of SARS-CoV-2 structural protein induced adventitial fibroblast inflammation, thrombosis, and diabetes progression. SARS-CoV-2 that has entered the vasculature can localize to the endothelium via gC1qR or transmigrate into the sub-endothelial space where viral structural proteins (Spike, Nucleocapsid, Membrane and/or Envelope Proteins) can interact with adventitial fibroblast gC1qR. The interaction of SARS-CoV-2 structural proteins with fibroblast gC1qR may activate internal signal transduction pathways (such as the MAPK pathway), which can lead to the enhanced expression or activation of GLUT-4, RAGE (receptor for advanced glycation end products), tissue factor, ICAM-1, and gC1qR's high molecular weight kininogen (HK) binding site. As each of these receptors interact with their specific ligands, vascular responses such as hypoglycemia, chronic inflammation, edema, coagulation, and thrombosis may be observed or promoted leading to vascular dysfunction. Further, each of these receptors and their heightened responses have been implicated in vascular disease progression. Importantly, these vascular disease responses have been shown to be heightened during COVID-19 progression, thus this figure proposes a possible mechanism to link SARS-CoV-2 infection and vascular disease progression.

conditions, the enhanced expression of any of these receptors would further promote or accelerate disease progression. Under healthy conditions, the expression of these receptors would predispose individuals to future vascular complications. It has been reported that patients who present with COVID-19, without underlying vascular complications, have altered vascular responses and for those that present with COVID-19 and vascular complications, vascular disease progression is significantly enhanced. Filling the gaps of our working hypothesis (Fig. 4) is essential to effectively manage COVID-19 progression.

Declaration of Competing Interest

The authors (BG) receive royalties from the sale of monoclonal antibodies against gC1qR clone 60.11 and clone 74.5.2. The authors hold a patent for the development of these antibodies for therapy against cancer and angioedema, respectively (US patent 8,883,153-B2, "Methods for Prevention and Treatment of Angioedema").

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