GAPDH, Interferon γ, and Nitric Oxide: Inhibitors of Coronaviruses

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As the COVID-19 pandemic finishes its second year, progress has been made against SARS-CoV-2 with vaccine candidates showing efficacy against this latest coronavirus strain. However, this pandemic presents a unique opportunity to investigate anti-viral therapies given the likely probability of another outbreak. One possible (and perhaps unlikely) therapeutic target could be GAPDH (glyceraldehyde-3-phosphate dehydrogenase). Studies have show that downregulation of GAPDH leads to a decrease in interferon gamma (IFNγ production which is an important cytokine response against coronaviruses and viruses in general). In this light, the previous coronavirus strain (SARS-CoV) has actually been shown to downregulate GAPDH. Although perhaps better known for its role in glycolysis, GAPDH also plays a role in gene expression of a varied set of genes by binding to their mRNA to affect stability and thereby translation. Moreover, GAPDH is also upregulated by nitric oxide (NO), an inhibitor against both SARS-CoV and SARS-CoV-2. Additionally, GAPDH has also been shown to be a negative transcriptional regulator of AT1R (angiotensin II receptor 1), which has been shown to bind ACE2 for eventual endocytosis of the complex implicating GAPDH’s potential role in the kinetics of coronavirus entry as well in downstream inflammatory signaling resulting from AT1R activation. Lastly, another important role for GAPDH is its requirement in the assembly of the GAIT complex that is responsible for termination of translation of IFNγ-responsive genes that would be critical for the resolution of any inflammatory response. These observations would imply that sufficient levels of GAPDH are needed for immune responses to function properly during a coronaviral infection. By examining different coronavirus studies, this review explores GAPDH’s role as an inhibitor of coronaviruses (at the viral transcriptional level and also as a modulator of gene expression related to inflammation), and its signal transduction links to the IFNγ and NO pathways.

Keywords: GAPDH, interferon gamma, nitric oxide, anti-viral, coronavirus, COVID-19

VIRAL TRANSCRIPTION

A number of recent studies have focused on the examination of host proteins in terms of their potential pro- and antiviral properties against coronaviruses (1–9). Yet, prior to these studies, the inhibitory role of GAPDH against coronavirus was demonstrated in a study that used the transmissible gastroenteritis coronavirus [TGEV belongs to the alpha subfamily of coronaviruses; SARS-CoV-2 which is responsible for the COVID-19 pandemic, and its predecessor, SARS-CoV, are in the beta family of coronaviruses (10)] to help identify proteins required for RNA transcription of the coronavirus virome (11). An RNA affinity chromatography pulldown identified hnRNPs...
(heterogeneous ribonucleoproteins), glutamyl-prolyl tRNA synthetase (EPRS) and poly(A)-binding protein (PABP) as candidates. As proof of concept, siRNA against those genes along with a GAPDH (typically thought of as a housekeeping gene) control was used. Afterwards, the host cells were infected with equivalent titers of the virus and qPCR for mRNA7 (responsible for encoding the nucleocapsid (N) protein, that encapsulates viral RNA and nonstructural proteins or nsp's) was quantified as shown in Figure 1. As expected, knockdown of the genes of the proteins from the pulldown resulted in decreased mRNA7 levels indicating their requirement for viral transcription.

The unexpected finding concerned the results from the GAPDH knockdown. By downregulating expression of this gene, the study found consistent upregulation (a 3-fold increase using 293T cells and a similar 2-fold increase using endothelial Huh-7 cells over controls) of mRNA7.

This would indicate that GAPDH couldn’t serve as a valid negative control, but furthermore, needed to be considered an experimental factor with a potentially important contribution to TGEV transcription. A possible explanation cited by the authors points to the example of GAPDH binding to the 3′ end of the cap protein of Hepatitis A virus (belonging to the family of picornaviruses) and destabilizing the internal ribosomal entry site (IRES structures are RNA elements that allow the virus to translate its virome) (12). Additionally, among coronaviruses, communication occurs between the 5′ and the 3′ ends of the viral RNA (which incidentally is around 30 kb and sometimes called a mini-genome due to the relatively large size for a virus) whereby the 3′ end directs the RNA synthesis or translation at the 5′ end. Since there are no complimentary sequences at either the 5′ or the 3′ ends to allow for direct interaction, the virus needs to recruit host proteins [such as the aforementioned ones found in the Galán et al., study] to serve as a bridge between the two viral genomic ends in order to facilitate transcription (13). In this light, it should also be noted that GAPDH is known to bind to AU-rich regions of certain RNA species (14) resulting in either stabilization or destabilization of mRNA and/or in regulation of translation (15). Lastly, another possible explanation is GAPDH has been shown to interact with an isoform of PABP, PABPN1 (16), which is important for RNA processing (17) and influenza viral transcription (18). Additionally, coronavirus gene expression was shown to be regulated by the interactions of the poly(A) tail, the coronavirus nucleocapsid and PABP proteins (19) which taken together compliment another study showing the requirement of PABN for coronavirus replication (20). These observations seem to point to the fact that GAPDH (perhaps through its interaction with PABP) plays a role in coronaviral transcription.

Furthermore, GAPDH levels were shown to decrease dramatically starting at 6-h post-infection in 293 cells infected with the SARS-CoV (the strain responsible for the 2003 outbreak) as compared to controls (21). Coupled with the Galán et al. study, a decrease in GAPDH levels upon infection would by logic lead to an expected increase in viral transcription, thereby implicating GAPDH as an inhibitor of coronaviruses (22). In addition, another study (23) was also able to demonstrate decreased levels of GAPDH (along with a decreased IFNβ expression) resulting from cells infected with the SARS-CoV. Moreover, this study showed that by specifically mutating the nsp1 gene and then re-infecting 293T cells, GAPDH levels remained unaffected which was simultaneously accompanied by a more robust anti-viral IFNβ response as compared to infection with a virus containing a wildtype nsp1 gene. Additionally, the study also observed a much more robust expression of ISG15 and ISG56 [which are involved in critical antiviral responses downstream of IFN I signaling (24)] infected with the virus with this mutant nsp1. The observation of a simultaneous decrease in both GAPDH and IFN I levels after SARS-CoV infection has been observed by other groups (25, 26) which would point to the nsp1 gene being a critical factor contributing to coronavirus virulence (27). Similarly, it has been shown that GAPDH is also dysregulated during SARS-CoV-2 infection. Logically, it would make evolutionary sense that SARS-CoV-2, whose genome is ~90% identical to that of the SARS-CoV strain (28), to turn down the host’s anti-viral response while simultaneously trying to make viral replication more efficient.

**IFNγ PATHWAY**

The overall explanation for the decreased GAPDH expression is that the coronaviruses turn down expression of the host’s endogenous genes non-specifically (26). However, there are reasons as to why the downregulation of GAPDH might be more consequential for the host cell’s anti-viral response. For example, the IFNγ pathway is one such response key to host defenses in general [for review, see (29)] including against coronaviruses. This can be seen in experiments where pre-treatment with IFNγ dramatically reduced TGEV viral replication (30, 31).

Aside from animal studies, it has been shown that patients who presented with acute respiratory distress syndrome (ARDS) due to a severe case of SARS-CoV infection had a decreased IFN (both alpha and gamma) response, which included the lack of accompanying downstream cascades of IFN-inducible genes,
in contrast to patients with milder cases (32). This is mirrored in the current COVID-19 pandemic where studies have found IFNγ levels to be downregulated in patients suffering from more severe cases of COVID-19 infection based on recent clinical data (33, 34). Another study showed that after stimulation with SARS-CoV peptides, IFNγ was the predominant cytokine secreted by memory T cells in patients (35). Taken together, these studies indicate that IFNγ responses are a crucial anti-viral mechanism for the host that become compromised in patients with more severe coronavirus infections.

In this light, it has been shown that proper IFNγ secretion actually requires sufficient levels of GAPDH. By stimulating macrophages with the addition of MPO (myeloperoxidase) to recapitulate inflammation, the study found that reducing GAPDH expression through RNAi resulted in a surprising 66% reduction in IFNγ secretion as compared to controls (36). This finding was further corroborated by another study that demonstrated that using an inhibitor of GAPDH, 3-bromopyruvic acid, blocked IFNγ synthesis (37). One possible explanation could be that nuclear GAPDH translocation (due to nitric oxide signaling which is discussed later) can increase CREB activity (38), which studies have shown to upregulate IFNγ at the transcriptional level (39, 40).

It should also be noted that further downstream, IFNγ has been shown to downregulate the ACE2 receptor, the host cell’s receptor to which the SARS coronaviruses attach (41). Pretreatment of Vero E6 cells with IFNγ (or IL4) significantly reduced infectious rates of the SARS-CoV through decreased ACE2 expression. Interestingly, while IL4 had no effect on the effect of IFNγ, pretreatment with IFNα had no effect on ACE2 levels indicating interferon species preference. However, a recent finding showed that ACE2 was upregulated following treatment with either IFNα2 and IFNγ (42) thereby ignoring a debate whether increased expression of the ACE2 receptor is advantageous for the virus or tissue-protective for the host (43). Another possibility could be a temporal aspect of ACE2 expression as it relates to interferon signaling which would need further experimentation. Incidentally, it may be worth mentioning that a broad set of cells are capable of being infected with SARS-CoV-2 virus (44) including monocytes.

Furthermore, there are other studies showing GAPDH’s role in the activation of the immune system (45), stabilization of colony stimulating factor 1 in macrophages (46), its extracellular secretion upon exposure to pathogens and binding to plasminogen and fibrinogen (47) as well as having other immunomodulatory roles (48) including stimulation of Type I Interferons (49). While this review hints at some of the contributions GAPDH likely plays in the host’s anti-viral mechanisms, the exact contribution of GAPDH to the pathogenesis of the current SARS-CoV-2 virus needs to be examined specifically (e.g., in vitro siRNA experiment similar to the Galán et al., paper using the SARS-CoV-2 virus instead).

**GAIT COMPLEX**

Another possible downstream consequence of GAPDH downregulation by coronaviruses can be found in the GAIT complex (interferon gamma inhibitor of translation) which consists of a quaternary complex of proteins that bind to a specific secondary stem-loop RNA structure (called the GAIT element) to terminate translation of inflammatory genes. In monocytes (e.g., macrophages, neutrophils, etc.), the GAIT element resides on IFNγ-responsive genes (e.g., VEGFa, CCL22, ceruloplasmin, etc.) which constitute the downstream cascade of a host cell’s IFNγ response (50). Interestingly, the GAIT complex was also recently shown to assemble in epithelial cells (51).

The order of binding is sequential with GAPDH binding last (after EPRS, NSAP1, and L13a) in order to terminate the translation of these crucial pro-inflammatory genes as shown in Figure 2 (52). As an example of the importance of the GAIT complex in downregulating IFNγ-responsive genes, studies have

![Image](https://example.com/image.png)

**FIGURE 2** | Order of assembly of the GAIT complex at specific stem-loop RNA structures (called the GAIT element) in IFNγ-responsive genes (52). With the presence of IFNγ, EPRS and NSAP1 assemble initially while L13a and GAPDH are the last proteins to assemble to enable binding to the the GAIT element present on the mRNA in order to terminate translation of the specific downstream genes.
shown that one such gene, ceruloplasmin, is crucial in order to elicit an inflammatory response in monocytes during infection. Ceruloplasmin synthesis can create an inhospitable environment for invasive pathogens due to its ferroxidase activity (53), while being crucial to elicit an inflammatory response, ceruloplasmin would then need to be downregulated in order to resolve the inflammatory process. This would require an intact GAIT complex that would presumably need sufficient levels of GAPDH in order to terminate the translation of this protein (and other pro-inflammatory proteins that are IFNγ-responsive).

As discussed, SARS-CoV downregulates GAPDH expression upon infection such that one could imagine a scenario that lacking sufficient levels of this crucial (i.e., last step) protein, the complex is unable to terminate anti-inflammatory responses in monocytes thereby leading to an unabated inflammatory response. In addition to downregulating GAPDH at the transcriptional level, one study has demonstrated that the TGEV coronavirus genome contains a stem-loop structure at the 3’ end that is capable of binding EPRS and functions as a true GAIT element in its ability to affect transcription of IFNγ-responsive genes as evidenced in the study (54). This would imply that this “decoy” GAIT element likely sequesters the GAPDH protein (in forming in the GAIT complex) thereby removing this protein from the available pool to perform other physiological functions. By examining the nucleotide sequence of the current SARS-CoV-2 virus, the database (https://www.ncbi.nlm.nih.gov/nuccore/NC_045512.2) lists three putative stem-loop structures at the 3’ end that could be potential GAIT elements: 29609..29644, 29629..29657, 29675..29903, and 29728..29768.

In addition to its role in controlling downstream IFNγ-responsive genes, it should also be noted that GAPDH can also bind to TNFα mRNA in macrophages to suppress translation until proper signaling (e.g., LPS stimulation) leads to malonylation of a critical residue releasing GAPDH to allow translation (55). This mechanism confirms an earlier study highlighting GAPDH’s role in TNFα expression (56). Besides regulating TNFα, a preclinical model of ARDS showed that pre-injecting GAPDH decreased levels of IL6 and resulted in a survival rate of 80% as opposed to a 100% mortality in control mice (57).

**ANGIOTENSIN PATHWAY**

Already mentioned in this review is GAPDH’s regulation of IFNγ. However, GAPDH is also a transcriptional regulator of a varied set of genes [for review, see (15)] that includes IFNγ and AT1R, the angiotensin II type 1 receptor. One function of AT1R is to bind to ACE2 (the host receptor for both the SARS-COV and SARS-Cov-2 viruses) leading to endocytosis of the complex into the cell (58, 59). GAPDH binds at the 3’ end of the AT1R mRNA acting as a negative transcriptional regulator. As such, knockdown of GAPDH resulted in a 2.5-fold upregulation of AT1R in HEK293 cells (60). Since we know from previous studies like Kamitani et al. that coronaviruses downregulate GAPDH, it would be expected that AT1R levels would be expected to increase upon infection. So, how would an increase of AT1R exactly help/be advantageous for coronaviruses? Changing the amount of expressed AT1R will likely affect the kinetics of ACE2 (some of which will be bound to the virus in SARS-CoV-2 patients) endocytosis and viral entry. It should also be noted that increased AT1R expression has been shown to result in inflammation and fibrosis (61).

**NITRIC OXIDE PATHWAY**

It is apparent that coronaviruses seem to have acquired multiple mechanisms to disable our immune system rendering some of us more susceptible to the onslaught of the virus. Having a glycolytic enzyme that is responsible for different (and vital) functions may be logical in an evolutionary perspective, but, at the same time, it can be a potential weakness that seems to be exploited by coronaviruses. As such, if GAPDH does prove to play a crucial role in the coronavirus life cycle and its subsequent pathogenesis, this protein could then be exploited as a therapeutic target. There are studies that have demonstrated different ways to raise cellular GAPDH concentrations (62) including stimulating nitric oxide (NO) synthesis—perhaps by using pharmaceutical therapies like...
inhaled NO (63). Physiologically, the nitric oxide synthetase (NOS) family of proteins that includes iNOS (inducible), eNOS (expressed in endothelial cells), and nNOS (expressed in neuronal cells) is responsible for the production of NO. More specifically, eNOS has been shown to be responsible for overall endothelial homeostasis whose dysfunction leads to pathologies like vascular inflammation and atherosclerosis (64). In COVID-19 patients suffering from ARDS, eNOS levels were significantly lower as compared to COVID-19 patients not suffering from ARDS (65). It has been proposed that in COVID-19 patients, inflammation associated with ARDS leads to the uncoupling of the eNOS activity which then leads to an imbalance in pulmonary vasoconstriction/vasodilation signaling pathways (66–68) as well as changes in the lung parenchyma. Lastly, the NO normally produced from eNOS activity helps vasodilate the blood vessels thus helping to prevent thrombus formation. This helps to explain the overall increased coagulopathy in COVID-19 patients with lower eNOS activity (69). In this light, a recent study has shown inhaled NO as being an effective respiratory strategy for COVID-19 patients (70).

Similarly, iNOS activity (responsible for antiviral activity) has also been shown to have a role in the pathology of SARS-CoV-2 infections. iNOS helps immune cells respond to pathogens and can be induced by lipopolysaccharides and cytokines (71). In infected cells, NO has been shown to nitrosylate the SARS-CoV-2 proteases (responsible for posttranslational processing of viral proteins)—papain-like protease (PLPRO) and chymotrypsin (MPRO)—as possible mechanisms that lead to an inhibition of SARS-CoV-2 (72, 73). Another possible mechanism includes nitrosylation of host proteins, namely cathepsins CatB and CatL, that have been shown to have a role in SARS-CoV-2 entry (74, 75). As such, it has been proposed that lower amounts of NO from reduced eNOS and/or iNOS activity impacts the pathology of COVID-19 infections in patients (76) which would suggest that NO could serve as a therapeutic target (77–80).

Overall, the use of NO dramatically slows down the replication of SARS-CoV in vitro (81, 82) as seen in Figure 3 which now have recently been corroborated using the SARS-CoV-2 strain also (83). Previously, it was shown that inhibitors of NO blunted the anti-viral activity of IFNγ in macrophages as evidenced by the restoration of viral replication in cells infected with vaccinia, ectromelia or herpes simplex-1 (84) using these inhibitors.

In order to induce NO synthesis, the Akerström et al. study also demonstrated that cells required incubation with IFNγ and IL1b. Other studies have shown upregulation of NO using requiring exposure to both IFNγ and TNFα in endothelial cells (85) as shown in Figure 4 or simply to IFNγ alone in neurons (86), and macrophages (87–90). Overall, iNOS levels were shown to peak between 12 and 24 h accompanied by a dramatic upregulation of GAPDH. Treatment with L-NMMA (Nω-[imino(methylamino)methyl]-L-ornithine, an inhibitor of NO synthesis) was shown to decrease both iNOS and GAPDH levels substantially (85). Taken together, these results indicate that NO production has a downstream stimulatory

![Figure 5](image.jpg)
effect on GAPDH levels, an observation that has since been confirmed (91).

Additionally, NO production can also lead to a nitrosylated form of GAPDH, thereby allowing translocation to the nucleus to perform non-glycolytic functions such as RNA export (92). Incidentally, NO has also been shown to increase expression of Herc6, an E3 ubiquitin ligase (91) in rat retinal ganglion cells. In mouse studies, Herc6 has been shown to stimulate the IFNγ promoter [likely through its actions on IRF3 (93) as well as through participation in the conjugation of ISG15 to viral targets for degradation (94)]. It should be noted that there appears to be a feedback loop whereby GAPDH can increase iNOS activity (95) by inserting a heme group into the iNOS protein. Lastly, a recent study showed that deficiency in both iNOS and another protein (IRG1, immune-responsive gene 1) resulted in a decreased IFNγ response and an inability to control pathogen replication (96). These observations point to an interrelationship between GAPDH, IFNγ production, and NO signaling which is summarized in Figure 5.

RELATED COVID-19 CLINICAL OBSERVATIONS

Lastly, how might the immunological consequences of GAPDH being downregulated by SARS-CoV (and likely SARS-CoV-2) be reflected in some of the current clinical presentations which we see in the COVID-19 pandemic? Statistics show that the earliest brunt of the mortality (before the vaccine rollout) was on elderly patients (97). This could reflect (or at least partly) the fact that GAPDH levels markedly decrease with age (98, 99). According to the Yamaguchi et al. study, decreasing GAPDH levels as observed among older patients would imply decreased IFNγ production which would impair the patient’s immune system’s ability. This was highlighted in a recent study demonstrating that adults are more susceptible to SARS-CoV-2 due to decreased IFNγ levels (100). It is also noteworthy that both NO (101) and interferon (102) production decrease with age.

Another group of studies (103, 104) also showed that females are far more likely to survive COVID-19 infection than males (by a ratio of 2:1). This was also seen in patients that were infected with Middle East Respiratory Syndrome-MERS coronavirus (105). A recent transcriptomic analysis of our immune system showed little differences between genders with the exception of macrophages which show sexual dimorphism (i.e., stronger immune response in females) after activation of the innate immune system and particularly after interferon stimulation (106). This would fit into the hypothesis that the lack of interferon response likely contributes to the pathology of coronaviruses.

In terms of comorbidities, it’s logical to see how a multifaceted protein like GAPDH might explain the COVID-19 statistics of those that are suffering from underlying conditions like diabetes. Reducing GAPDH levels, as we have seen coronaviruses do, would decrease the amount of GAPDH available for serving vital roles in these patients (whose systems are already being taxed) that depend on its vital glycolytic function to help maintain optimal health (107). Perhaps, one can think of this as being analogous to two-hit hypothesis (108) in cancer whereby the virus becomes the second hit, and the source of morbidity being the first hit (“health dysfunction”) because as the virus decreases GAPDH levels, the cells in these patients are likely struggling to maintain adequate homeostatic levels leading to a physiological tug-of-war.

CONCLUSIONS

In addition to nitric oxide, another therapeutic compound, dexamethasone (a corticosteroid), which has been shown to increase GAPDH levels (109, 110), has also proven effective against SARS-CoV-2 (111). Lastly, if the feedback loop in Figure 5 is correct, measuring a patient’s relative GAPDH levels may be useful for clinicians since differences in expression levels can be further amplified even further in signaling pathways downstream. The fact that many of the studies reviewed here centered on monocytes (macrophages in particular) underscores the importance of the innate immune system against coronaviruses. While some of the findings reviewed here center on SARS-CoV-2 directly, other results come from other members of this family of viruses given the lack of information on this latest coronavirus strain. Pieced together, they point to the GAPDH/IFNγ/NO pathways as potential therapeutic targets against coronaviruses that should be examined in greater detail. By knowing these molecular steps, anti-viral strategies like NO therapy could perhaps be better utilized to fight this current (and future) pandemic(s).

AUTHOR CONTRIBUTIONS

AA conducted literature search and wrote the manuscript.

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