Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Trends in Immunology

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Opinion

NAD^+ in COVID-19 and viral infections

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NAD^+, as an emerging regulator of immune responses during viral infections, may be a promising therapeutic target for coronavirus disease 2019 (COVID-19). In this Opinion, we suggest that interventions that boost NAD^+ levels might promote antiviral defense and suppress uncontrolled inflammation. We discuss the association between low NAD^+ concentrations and risk factors for poor COVID-19 outcomes, including aging and common comorbidities. Mechanistically, we outline how viral infections can further deplete NAD^+ and its roles in antiviral defense and inflammation. We also describe how coronaviruses can subvert NAD^+-mediated actions via genes that remove NAD^+ modifications and activate the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome. Finally, we explore ongoing approaches to boost NAD^+ concentrations in the clinic to putatively increase antiviral responses while curtailing hyperinflammation.

Highlights

NAD^+ can exert both antiviral and anti-inflammatory effects in mice and humans, which might be beneficial during coronavirus disease 2019 (COVID-19) infections.

Compared with healthy individuals, NAD^+ concentrations in tissues and organs are lower in older individuals and in patients with diseases associated with severe COVID-19 symptoms, including diabetes and cardiovascular disease.

Viral infections, including coronavirus infections, have been reported to further deplete cellular NAD^+ stores.

Many NAD^+-dependent enzymes, including members of the sirtuin and poly-ADP-ribose polymerase (PARP) families, display potent antiviral activities.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and a variety of viruses possess ADP-ribosyl hydrolase activity, which counteracts the activity of PARPs.

Viruses such as SARS-CoV-2 can hyperactivate the immune system by activating nuclear factor kappa B (NF-κB) and the NOD-, LRR-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, potentially leading to deadly cytokine storms. NAD^+-boosting compounds may counteract these processes.

Several NAD^+-boosting compounds and molecules that target NAD^+-producing or -consuming enzymes are in clinical development as putative anti-inflammatory or antiviral drugs.

NAD^+ as a modulator of viral infection outcomes

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other viral infections render the body a battlefield, requiring mobilization of vast resources to mount an effective defense. These defenses can backfire when uncontrolled, resulting in deadly **cytokine storms** (see Glossary). A key to effective interventions is to trigger robust antiviral defenses but with checked inflammation. Therefore, molecules that suppress both viral replication and inflammation may be particularly important for fighting severe COVID-19 [1]. A growing body of evidence shows that the metabolite NAD^+ is a mediator of both antiviral and anti-inflammatory mechanisms. Based on this evidence, we posit that therapies that boost NAD^+ concentrations might play a role in preventing and treating severe COVID-19 and other viral infections.

First described as a yeast fermentation factor over a century ago, today, NAD^+ has risen to prominence as a regulator of healthy aging. Low levels of NAD^+ in tissues and organs are associated with aging, metabolic syndrome, and inflammation, while dietary interventions that slow age-related diseases increase NAD^+ concentrations [2]. Here, we review the epidemiological and mechanistic data supporting a role for NAD^+ in modulating the outcomes of viral infections, with a focus on SARS-CoV and SARS-CoV-2. We also explore ongoing approaches to boost NAD^+ levels for therapeutic benefit in the clinic.

NAD^+ and risk factors for severe COVID-19

Aging

A tragic and poorly understood aspect to COVID-19 is the increased susceptibility of the elderly to severe forms of the disease [3]. Rates of hospitalization, intensive care unit (ICU) admission, and death increase with age, while the young are often left relatively unscathed [4]. Most infectious diseases afflict both the very old and young, making this demographic pattern for COVID-19 susceptibility unusual [5]. A better understanding of the mechanisms that render older individuals more susceptible to developing severe disease could lead to new candidate strategies for therapeutic intervention.

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We posit that one culprit may be a deficiency in NAD^+, the concentration of which decreases during aging in nearly every tissue studied across species [6]. In rodents, this decrease has been observed in tissues and cells that are relevant to COVID-19 infection, such as the skeletal muscle, liver endothelial cells, and macrophages [7–10]. A growing body of human data corroborates an association between age and low NAD^+ concentrations across multiple tissues including the skin, blood, liver, and muscle [10–13,117].

Why NAD^+ levels fall during aging is an active area of research. Studies in mice have implicated an increase in the concentrations and activity of NAD^+-consuming enzymes such as CD38, **poly (ADP-ribose) polymerase** 1 (PARP1)1, and sterile alpha and TIR motif containing 1 (SARM1) [9,14–17], as well as an insufficient flux from NAD^+-producing enzymes such as nicotinamide phosphoribosyltransferase (NAMPT), indoleamine 2,3-dioxygenase (IDO), and quinolinate phosphoribosyltransferase (QPRT) [10,18–20]. These enzymes all serve as potential pharmacological targets to raise NAD^+ concentrations (Figure 1).

Comorbidities

NAD^+ concentrations are also low in certain comorbidities associated with COVID-19 severity. One such comorbidity is insulin resistance (IR) and diabetes mellitus [21]. Specifically, in mouse models, genetic (KK/HIJ), diet-induced (high-fat diet), streptozotocin-induced, and age-associated IR have been associated with lower NAD^+ concentrations in liver and white adipose tissue, while restoration of NAD^+ with NAD^+ boosters such as nicotinamide ribose (NR) and nicotinamide mononucleotide (NMN) (oral, intraperitoneal, or infusion) reverses IR [22–26]. In humans, metabolic syndrome and obesity have been associated with low NAD^+ concentrations in adipose tissue [27]. NMN was recently shown to improve insulin sensitivity in prediabetic women, and further clinical studies are ongoing, discussed later in this review [28] (Table 1, Key table). In addition, a recently developed oral antidiabetic medication, **imeglimin**, can enhance glucose-stimulated ATP generation and induce the synthesis of NAD^+ in pancreatic islets derived from diseased rodents with type 2 diabetes [29].

Another risk factor for severe COVID-19 is cardiovascular disease (CVD) [30]. Several preclinical studies demonstrate that raising NAD^+ concentrations in endothelial cells reverses vascular and endothelial dysfunction [8,31]. Further studies have shown a beneficial role for NAD^+ in cardiovascular function in mouse models, including models of dyslipidemia, ischemia–reperfusion injury, and diastolic heart failure [32,33]. This link is supported in humans by epidemiological data demonstrating a correlation between dietary intake of the NAD^+ precursor niacin (nicotinic acid) and vascular health, including brachial-artery flow-mediated dilation and serum low-density lipoprotein (LDL) concentrations [34]. In fact, niacin is an USA FDA-approved therapy for reducing LDL, ApoB, and triglycerides, and for raising high-density lipoprotein (HDL). Additionally, multiple ongoing clinical studies are testing the effects of other NAD^+ boosters such as NMN and NR on hypertension and heart failure (discussed later) (Table 1).

Mechanisms of NAD^+ in viral infections

Viral infections can deplete NAD^+ concentrations

Not only are low NAD^+ concentrations associated with risk factors for poor COVID-19 outcomes, but certain viral infections can further deplete NAD^+ in infected cells. For example, lower NAD^+ concentrations have been reported in human peripheral blood leukocytes infected with HIV-1 *in vitro* [35], human fibroblasts infected with herpes simplex virus 1 (HSV-1) [36], and in the skeletal muscle of individuals coinfected with HIV-1 and hepatitis C virus [37]. These effects are presumably due to the induction of NAD^+-consuming enzymes such as CD38 and PARPs [17]. For instance, $CD8^+$ T lymphocytes expressing CD38 have been proposed as a marker for HIV-1-mediated disease

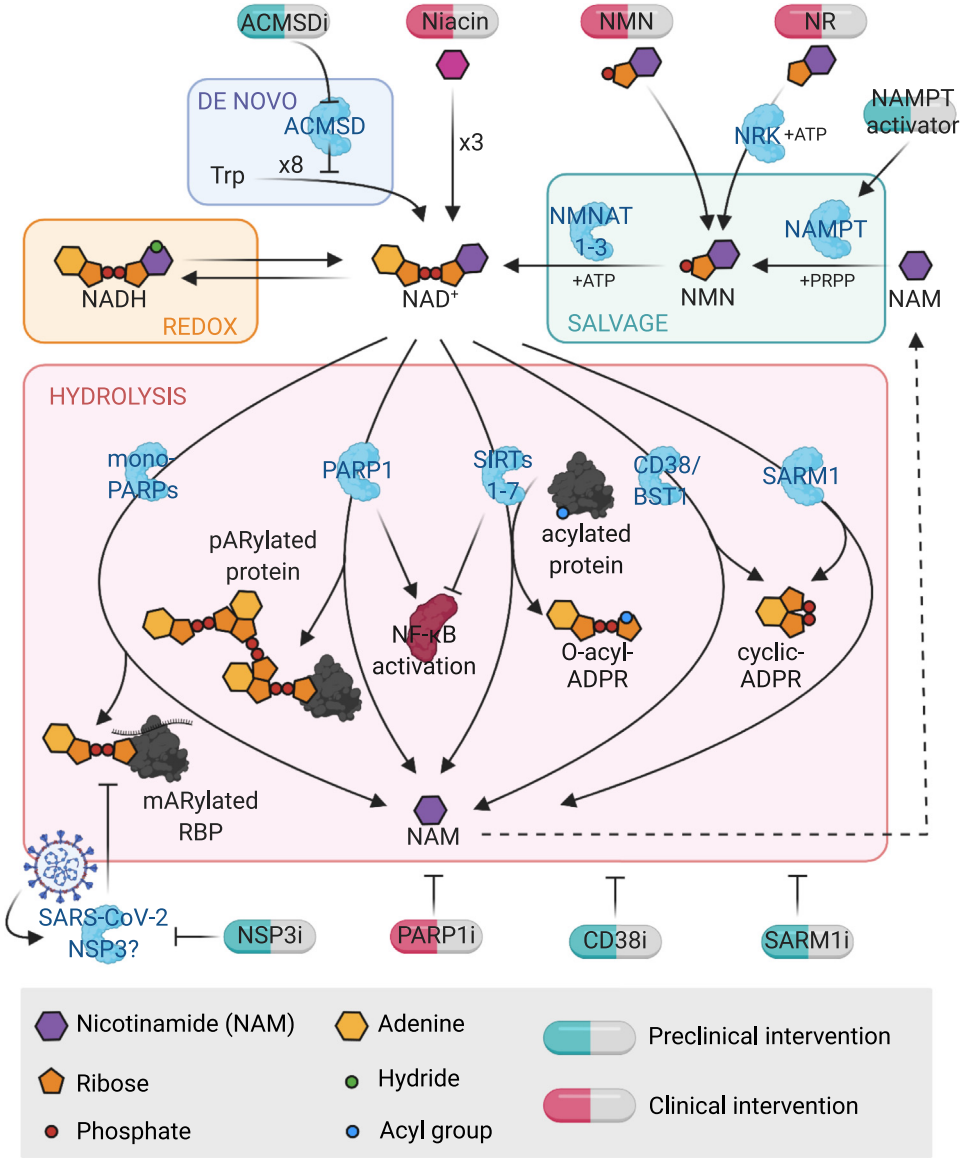

Figure 1. NAD$^+$ metabolism and points of pharmacological intervention. Enzymes involved in NAD$^+$ biosynthesis and hydrolysis play important roles in inflammation and immunity. Biosynthetic pathways include the NAD$^+$ salvage pathway, which recycles nicotinamide to form NMN, then NAD$^+$, and the *de novo* pathway that begins with tryptophan. Hydrolysis of NAD$^+$ is largely carried out by PARPs, which tag target proteins with poly- or mono-(ADP ribose); sirtuins, which remove acyl groups and create O-acyl-ADP-ribose; and CD38, BST, and SARM1, which create (cyclic)-ADP-ribose [44]. There are multiple points of potential pharmacological intervention throughout NAD$^+$ metabolic pathways. Created with BioRender.com. Abbreviations: i, inhibitor; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; NR, nicotinamide ribose; NSP3, nonstructural protein 3; PARP, poly(ADP-ribose) polymerase; SARM1, sterile alpha and TIR motif containing 1; SIRT, sirtuin.

progression [38]. The decline of NAD$^+$ concentrations in human fibroblasts induced by HSV-1 infection is associated with increased protein poly(ADP-ribosyl)ation, and can be blocked by pharmacological inhibition of PARP1/PARP2 [36].

Glossary

Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC): inflammasome adaptor that plays a key role in the assembly and activation of the inflammasome. The NLRP3 inflammasome consists of a sensor (NLRP3), an adaptor (ASC), and an effector (caspase-1). Upon sensing-specific triggers, NLRP3 undergoes conformational changes which catalyze ASC oligomerization to form a macromolecular signaling complex known as the ASC speck. ASC then recruits caspase-1. Active caspase-1 cleaves pro-IL-1β and pro-IL-18, which are vital cytokines during infection and inflammation.

Cytokine storm: life-threatening event triggered by uncontrolled inflammatory responses with the release of a large amounts of proinflammatory cytokines, including IL-6, IL-1, TNFα, and IFN. This leads to the recruitment and expansion of various immune cells into injury sites which can result in tissue and organ damage.

Histone H3 lysine 9 (H3K9) deacetylation: epigenetic modification; H3K9 is generally acetylated when a gene is transcriptionally activated and is deacetylated (and/or methylated) when a gene is silenced.

Imeglimin: novel oral agent for the treatment of type 2 diabetes. Its mechanism of action involves amplification of glucose-stimulated insulin secretion and enhanced insulin action. Approved for use in Japan in 2021.

P2X purinoceptor 7: expressed in an increasing number of cell types; primarily mediates inflammation and cell death; ligand-gated cation channel activated by high concentrations of extracellular ATP, triggering the assembly and activation of the NLRP3 inflammasome and subsequent release of IL-1β and IL-18.

Poly(ADP-ribose) polymerases (PARPs): with coenzyme NAD$^+$, PARP enzymes catalyze ADP-ribosylation or the transfer of ADP-ribosyl groups from NAD$^+$ to nucleophilic side chains of proteins. They can also ADP-ribosylate DNA and RNA. In humans, there are 17 identified PARPs. PARPs participate in diverse cellular functions such as DNA repair, apoptosis, unfolded protein response, pathogen response, and inflammation.

Sirtuins: enzymes that couple NAD$^+$ degradation to deacylation (e.g., deacetylation, desuccinylation, and

Similar depletion of NAD^+ has been reported with coronavirus infections. Specifically, blood from severe COVID-19 patients contains lower amounts of the NAD^+ precursor NMN compared with blood from healthy individuals [39]. Moreover, expression changes in genes involved in NAD^+ synthesis and utilization such as *Nampt*, *Parp9*, *Parp12*, and *Parp14* have been observed in epithelial cell lines and enterocyte organoids infected with SARS-CoV-2 compared with mock infection [40]. Similar NAD^+-related gene expression changes were also reported in the lung tissue of a deceased COVID-19 patient and in the bronchoalveolar lavage fluid of SARS-CoV-2-infected individuals relative to healthy controls [40]. Furthermore, infection of a coronavirus, mouse hepatitis virus (MHV), in mouse bone-marrow-derived macrophages (BMDMs) induced NAD^+ depletion [40] as well as increased gene expression of many PARPs including *Parp7*, *Parp9*, *Parp10*, *Parp11*, *Parp12*, *Parp13*, and *Parp14* relative to mock infection [41]. These observations suggest that NAD^+ metabolic pathways are under increased demand during SARS-CoV-2 infection, and highlights the potential relevance of NAD^+ in modulating COVID-19 disease outcomes.

NAD^+-consuming enzymes in antiviral mechanisms

NAD^+ harbors an important role in fueling the activity of enzymes that regulate mammalian immune responses [42,43]. Classically, NAD^+ participates in redox processes, but it also participates in non-redox reactions in which it is hydrolyzed, and it non-enzymatically regulates protein–protein interactions [16] (Figure 1). In these latter functions, NAD^+ acts as a signaling molecule, serving as a marker of energy availability and directing a cell to respond to metabolic changes via the action of NAD^+-utilizing enzymes [44].

PARPs and **sirtuins** are two NAD^+-dependent enzyme families that participate in immune responses [42,43]. By adding or removing post-translational modifications on key proteins such as nuclear factor kappa B (NF-κB), they can coordinate the intensity of inflammatory and immune responses [42]. This places NAD^+ in an important position for both promoting strong immune responses to pathogens, and for keeping those responses in check.

PARP1, the pre-eminent member of the PARP family, is a potent coactivator of the proinflammatory transcription factor NF-κB, and therefore participates in initiating specific immune responses [42]. However, this is a double-edged sword, as PARP1 may also increase the severity of cytokine storms as it regulates the expression of many NF-κB-dependent cytokines and chemokines [42]. Indeed, inhibiting or deleting PARP1 has ameliorated the severity of symptoms in several inflammatory disease rodent models including asthma and colitis [45–51]. For example, PARP1 inhibition (pharmacological or genetic) has prevented ovalbumin-induced lung inflammation in mice [45] and in a guinea pig model of asthma [46]. Treatment with PARP inhibitors has attenuated inflammation associated with colitis seen in interleukin-10 (IL-10) deficient (*Il10*$^{-/-}$) mice [47], as well as in trinitrobenzene sulfonic acid-treated rats [49]. Furthermore, PARP1 knockout (KO) (*Parp1*$^{-/-}$) mice are protected from dextran sulfate sodium-induced colitis compared with wild-type mice [48].

Several PARPs harbor potent antiviral functions [42,52–59]. Indeed, PARP13 is a powerful antiviral factor that recognizes various viruses from several families, including Retroviridae, Filoviridae, Alphaviridae, and Hepadnaviridae [53–56]. It binds to specific sequences of viral RNAs during infection and mediates their degradation via the cellular mRNA decay machinery; however, these functions are not dependent on PARP-mediated ADP-ribosylation [54–56]. Expression of PARP7, PARP10, and the long isoform of PARP12 (PARP12L) efficiently inhibits cellular translation and the replication of Venezuelan equine encephalitis virus and other alphaviruses in vertebrate cells [52,59]. These effects of PARP12L are dependent on its catalytic activity. Moreover, PARP9 and PARP14 are also upregulated in macrophages stimulated by interferon (IFN)-γ and

demyristoylation). With NAD^+, sirtuins remove acyl groups from lysine residues on proteins, sense intracellular NAD^+ concentrations, and transduce a signal via protein deacylation. In humans, there are seven sirtuins at different cellular locations that regulate diverse functions, including transcription, genome stability, metabolism, and cell signaling.

Stress granules (SGs): dense, membraneless, organelles in the cytosol that appear in response to cellular stress to promote cell survival. Ribonucleoprotein assemblies that store mRNAs stalled at translation initiation. The introduction of viral RNA into the cytoplasm triggers the formation of stress granules.

TNF receptor-associated factor 3 (TRAF3): member of the TRAF family; contains a RING domain with E3 ubiquitin ligase activity, and a TRAF domain mediating protein–protein interactions; functions in inflammation, antiviral defense, and apoptosis. There are seven TRAF proteins identified in mammals.

Toll/interleukin-1 receptor domain: intracellular signaling domain in proteins that mediates protein–protein interactions between Toll-like receptors and signal transduction proteins. When activated, the TIR domain recruits cellular adaptor proteins and induces downstream activation of kinases.

Key table

Table 1. Selected clinical trials involving NAD^+ boosters

Clinicaltrials.gov ID	Phase	Interventions	Duration	Type	Enrollment (participants)	Inclusion criteria	Primary endpoint	Completion
NCT03151239	N/A	NMN, 250 mg/d or placebo	8 wk	Randomized, double-blind, placebo-controlled	25	Prediabetic postmenopausal women age 55–75 yr	Change in muscle insulin sensitivity	June 2021
NCT05175768	N/A	NMN, NMN +L-leucine, or placebo	Up to 28 d	Randomized, double-blind, placebo-controlled	375	Individuals age >40 yr hospitalized with COVID-19 requiring supplemental oxygen	COVID-19 associated fatigue	December 2022
NCT04903210	IV	NMN, 800 mg/d + lifestyle modification or lifestyle modification alone	8 wk	Randomized, single blind	20	Individuals age 18–65 yr with mild essential hypertension (BP 130/80–159/99)	Hypertension (flow-mediated dilation and brachial–ankle pulse wave velocity)	July 2022
NCT04664361	N/A	NMN 250 mg/d, NMN 500 mg/d, or placebo	38 d	Randomized, double-blind, placebo-controlled	150	Healthy men age 20–49 yr with regular moderate physical activity	Muscle recovery (post-endurance Wingate Anaerobic Test)	September 2022
NCT02950441	II	NR 1 g/d or placebo	21 d, followed by washout and crossover	Randomized, double-blind, placebo-controlled, crossover	12	Men age 70–80 yr	Mitochondrial function (respirometry) and NAD^+ concentrations in muscle biopsy	September 2019
NCT02921659	I/II	NR 1 g/d or placebo	6 wk, followed by crossover	Randomized, double-blind, placebo-controlled, crossover	30	Individuals age 55–79 yr	Treatment-emergent adverse events	October 2016
NCT04040959	II	NR 1 g/d or placebo	3 mo	Randomized, double-blind, placebo-controlled	118	Individuals age 35–80 yr with chronic kidney disease stage III or IV	Arterial stiffness (carotid–femoral pulse wave velocity)	September 2024
NCT03821623	II	NR 1 g/d or placebo	3 mo	Randomized, double-blind, placebo-controlled	118	Individuals age ≥50 yr with systolic blood pressure between 120 and 139 mmHg	Resting systolic blood pressure	December 2023
NCT04528004	I	NR dose escalation to 1 g/d or placebo	14 d	Randomized, double-blind, placebo-controlled	40	Adults with end-stage heart failure NYHA class IV	Whole blood NAD^+ concentrations	August 2024
NCT04407390	II	NR 1 g/d or placebo	14 d	Randomized, double-blind, placebo-controlled	100	Individuals age ≥70 yr with COVID-19	Hypoxic respiratory failure	May 2022
NCT04818216	II	NR 1 g/d or placebo	10 d	Randomized, double-blind, placebo-controlled	100	Adults hospitalized with COVID-19 and acute kidney injury	Whole blood NAD^+ concentrations, adverse events, thrombocytopenia	June 2023
NCT04573153	II/III	NR + serine + L-carnitine tartrate + N-acetylcysteine + hydroxychloroquine vs. placebo + hydroxychloroquine	14 d	Randomized, placebo-controlled	400	Adults with COVID-19, ambulatory and symptomatic	Hospitalization rate	March 2021

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Table 1. (continued)

Clinicaltrials.gov ID	Phase	Interventions	Duration	Type	Enrollment (participants)	Inclusion criteria	Primary endpoint	Completion
NCT04809974	IV	NR 2 g/d or placebo	22 wk	Randomized, double-blind, placebo-controlled	100	Individuals age 18–65 yr, 2+ mo out from COVID-19 PCR diagnosis, currently PCR negative, with persistent cognitive and physical difficulties (long-COVID)	Cognitive functioning measured by executive functioning and memory composite scores	December 2022

have opposing roles in macrophage activation [58]. PARP9 activates IFNγ–STAT1 signaling and induces proinflammatory activation while PARP14 ADP-ribosylation reduces STAT1 phosphorylation in IFNγ-treated human macrophages [58]. , Additionally, the nucleocapsid proteins of several coronaviruses, including SARS-CoV, MERS-CoV, and MHV, are ADP-ribosylated in infected cells, presumably by PARPs, which may indicate a common use of this pathway among viruses. However, the functional consequences of such ADP-ribosylation remain to be investigated [57]. We argue that since PARP enzymatic activity requires NAD^+[44], maintaining a sufficient NAD^+ concentration may be crucial for achieving PARP-related antiviral mechanisms.

Sirtuins also play a role in antiviral defenses [43,60–64]. Indeed, sirtuin 1 (SIRT1) KO or inhibition promotes the lifecycle and replication of vesicular stomatitis virus in mouse embryonic fibroblasts (MEFs) and Kaposi's sarcoma-associated herpesvirus in human lymphoma cell lines [61,62]. Disruption of SIRT1 also increases HPV16 E1–E2 replication [60]. Moreover, knockdown via siRNA of each of the seven sirtuins in human fibroblast cells promoted the growth of a diverse set of human viruses after infection, including human cytomegalovirus (CMV), HSV1, adenovirus type 5, and influenza virus (H1N1) [63]. Furthermore, SIRT1-activating drugs such as resveratrol and CAY10602 have inhibited the replication of these viruses [63]. SIRT6 promotes tumor necrosis factor (TNF)α secretion, as evidenced from the suppression of TNFα release from SIRT6 KO MEFs, whereby TNFα secretion would be expected to promote the eradication of pathogens [64]. Indeed, pharmacological inhibition and siRNA knockdown of SIRT6 and NAMPT, an NAD^+-synthetic enzyme (Figure 1), in mouse fibroblasts promoted CMV replication [65].

Other NAD^+-utilizing enzymes that also play roles during immune responses include CD38 [66], BST1 [2], and SARM1 [43]. Indeed, CD38 and BST1 are highly expressed on the surface of macrophages and lymphocytes and produce extracellular cyclic(ADP-ribose), a calcium-mobilizing second messenger that is important for immune cell activation [2,66,67]. SARM1, another potent NADase, contains a **Toll/IL-1 receptor domain**, which might elicit neuroprotective innate immune responses, as suggested from mouse models of neurodegeneration [68].

Viruses fighting back

One antiviral mechanism that is associated with PARP activity is the formation of cytosolic, membraneless organelles called **stress granules (SGs)** to sequester viral RNAs and arrest proviral translation [69,70]. Indeed, ADP-ribosylation of SG components by PARPs promotes the formation of antiviral SGs [71–73]. Several antiviral PARPs including PARP5a, 12, 13, 14, and 15 localize to SGs in human cells, presumably to execute this modification [71].

As a counter-mechanism to the host, many viruses, including coronaviruses and alphaviruses, deploy ADP-ribosylhydrolases to remove SG-promoting ADP-ribose modifications [74–77]. SARS-CoV-2 has such a domain with mono-ADP-ribosylhydrolase activity in its largest encoded protein, nonstructural protein 3 (NSP3) [79,80]. Ectopic expression of the SARS-CoV-2 NSP3 macrodomain reverses PARP9-dependent ADP-ribosylation of target proteins [81]. In live viruses, this domain is important for both viral replication and virulence. Mutations of this domain in MHV and SARS-CoV led to attenuated viral replication, and thus, the mutant viruses were unable to cause lung disease in infected mice, while treatment with pan-PARP inhibitors enhanced SARS-CoV replication and inhibited IFN production in BMDMs infected with macrodomain-deficient mutant coronavirus [41,82]. These findings suggest that the SARS-CoV-2 NSP3 protein might also interfere with SG formation and thereby allow for evasion of cellular antiviral responses (Figure 2). We argue that providing more NAD^+ to fuel the activity of PARPs might shift the antiviral balance back in favor of host cells.

Viruses can drive uncontrolled inflammation

One of the potential catastrophic effects of SARS-CoV-2 infections is cytokine storms. Indeed, the concentrations of circulating proinflammatory factors such as IL-1, IL-6, and TNFα are strongly associated with ICU admission and mortality in COVID-19 patients [83,84]. Studies of the first SARS pandemic coronavirus, SARS-CoV, have shown that viral proteins play an active role in the processing and release of the two specific proinflammatory cytokines, IL-1β and IL-18, by enhancing NF-κB transcriptional activity and promoting the formation of the NLRP3 inflammasome (Figure 2) [85–90].

The SARS-CoV proteins that facilitate these processes include open reading frame (ORF)3a, envelope protein (E), and ORF8b [85–90]. ORF3a mediates NF-κB activation through **TRAF3**-dependent ubiquitination of an NF-κB inhibitory subunit, and mediates speck formation of the inflammasome subunit **ASC**, which accompanies assembly of the NLRP3 inflammasome in human cells [86]. Additionally, ORF3a has transmembrane domains and ion channel (IC) activity that drives K^+ efflux, which further promotes activation of the NLRP3 inflammasome. Indeed, IL-1β secretion was completely blocked when BMDMs, stimulated with lentiviruses expressing the SARS-CoV ORF3a, were treated with K^+-rich medium [78,85]. The SARS-CoV E protein also promotes inflammasome activation through its intracellular activity; it forms lipid–protein channels at the endoplasmic reticulum (ER)–Golgi intermediate compartment, and the resulting Ca^{2+} stimulates the activation of the NLRP3 inflammasome. The E protein also promotes IL-1β release in mammalian cells expressing the NLRP3 inflammasome, while E protein mutants lacking ion conductance cannot boost IL-1β secretion [87,88]. ORF8b causes inflammasome activation in human macrophages and interacts directly with NLRP3 *in vitro*; moreover, it can form protein aggregates and trigger ER stress and lysosomal damage that activate the inflammasome in HeLa cells [89]. These processes not only promote virulence but may also facilitate viral replication [87,90]. For example, a comparative study of the functional motifs included within the SARS-CoV viroporins showed that full-length E and ORF3a proteins were required for maximal SARS-CoV replication and virulence in infected mice [87].

As with SARS-CoV, SARS-CoV-2 can also activate the inflammasome [91]. Active NLRP3 inflammasomes have been found in peripheral blood mononuclear cells (PBMCs) and tissues from deceased COVID-19 patients upon autopsy, along with higher concentrations of serum IL-18 which correlated with COVID-19 severity. SARS-CoV-2 may activate the inflammasome through mechanisms similar to those of SARS-CoV [91]. Overexpression of SARS-CoV-2 ORF3a in human cells can induce K^+ efflux, NLRP3 activation, and IL-1β release. Restricting K^+ efflux with K^+-rich media impairs the ability of ORF3a to trigger NLRP3 inflammasome

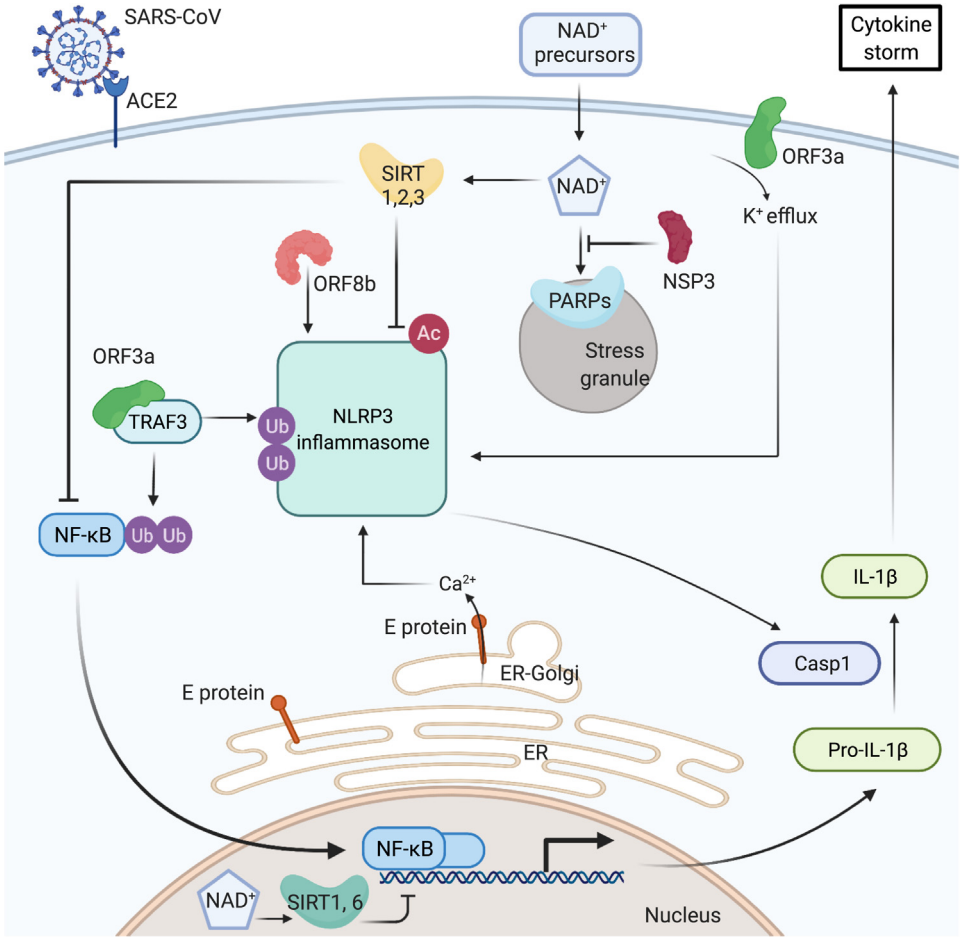

Figure 2. Regulation of the NLRP3 inflammasome by SARS-CoV infection and NAD^+. Several proteins encoded by SARS-CoV promote NLRP3 inflammasome activity and the release of proinflammatory cytokines. ORF3a activates NF-κB through TRAF3-dependent ubiquitination, which facilitates ASC speck formation and the assembly of NLRP3 inflammasome [86]. ORF3a also has transmembrane domains and ion channel activity that drives a K^+ efflux [85]. E protein is located at the ER–Golgi compartment and promotes Ca^{2+}efflux [87,88]. ORF8bdirectly interacts with NLRP3. These mechanisms all activate the inflammasome [89]. Host cell SIRT1, SIRT2, and SIRT3 all suppress the NLRP3 inflammsome [94–98]. SIRT1 deacetylates NF-κB, suppressing its activity, and reduces oxidative stress, decreasing inflammasome activation. SIRT2 deacetylates NLRP3. SIRT3 suppresses mitochondrial ROS production, decreasing inflammasome activation. SIRT6 reduces inflammation via H3K9 deacetylation in the promoters of NF-κB target genes [99]. PARPs promote antiviral SG formation through ADP-ribosylation of SG components [71–73]. Created with BioRender.com. Abbreviations: Ac, acetylation; ACE-2, angiotensin-converting enzyme 2; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain Casp1, caspase-1; ER, endoplasmic reticulum; NF-κB, nuclear factor κB; NLRP3, NOD-, LRR-, and pyrin domain-containing protein 3; ORF, open reading frame; SARS-CoV, severe acute respiratory syndrome coronavirus 1; SIRT, sirtuin; TRAF3, TNF receptor-associated factor 3; Ub, ubiquitination.

assembly, as evidenced from coimmunoprecipitation (co-IP) assays [92]. SARS-CoV-2 N protein can interact directly with NLRP3, supported by results of reciprocal co-IP and confocal microscopy, thus promoting inflammasome activation and IL-1β release in cells. In *Nlrp3*$^{+/+}$ mice infected with adeno-associated virus (AAV)-Lung-N, serum IL-1β concentrations were increased (detected via ELISA), whereas IL-1β was not induced by AAV-N in the sera of *Nlrp3*$^{-/-}$ mice [93]. The higher cytokine concentrations stemming from NLRP3 activation

suggest an increased likelihood of uncontrolled inflammation in response to SARS-CoV-2 infection.

NAD^+-consuming enzymes in anti-inflammatory mechanisms

NAD^+ may contribute to the resolution of inflammation, and to limiting or preventing the effects of cytokine storms; it might do so by increasing the activity of sirtuins (Figure 2) [44]. SIRT1, SIRT2, and SIRT3 all suppress the activity of NF-κB and the NLRP3 inflammasome via multiple mechanisms [94–98]. From a biochemical standpoint, SIRT1 physically interacts with and deacetylates NF-κB and thereby suppresses its transcriptional activity in human epithelial cells [95]. SIRT1 also inhibits lipopolysaccharide-induced NLRP3 inflammasome activation by reducing oxidative stress, as demonstrated in human trophoblasts with an shRNA knockdown of SIRT1 [94]. SIRT2 directly deacetylates NLRP3 and inactivates the NLRP3 inflammasome, as shown in SIRT2 KO (*Sirt2*$^{-/-}$) mouse macrophages [97]. SIRT3 mediates inflammasome activation by suppressing mitochondrial reactive oxygen species (ROS) production, which can activate the NLRP3 inflammasome. SiRNA depletion of SIRT3 in human macrophages results in increased ROS production and inflammasome activation compared with controls [98]. SIRT6 also promotes the resolution of inflammation via **histone H3 lysine 9 (H3K9) deacetylation** in the promoters of NF-κB target genes [99]. However, the effects of sirtuins on the NLRP3 inflammasome have yet to be extensively studied in the context of viral infection.

We posit that NAD^+ boosters that suppress NF-κB and NLRP3 inflammasome activity might also represent potential treatments to ameliorate the inflammatory symptoms of COVID-19. Indeed, treatment with NR, an NAD^+ precursor, for 24 hours has been reported to decrease the release of IL-1β and blunt inflammasome activation in PBMCs from healthy and fasted individuals [98]. NR also promotes SIRT1 expression, suppresses NLRP3 expression, and reduces secretion of proinflammatory factors TNFα and IL-6 in mouse hepatocytes [100]. Another NAD^+ precursor, nicotinic acid, also reduced inflammation in a rodent model of type 2 diabetes (KK/HIJ mice) by modulating NLRP3 activity [26]. However, consideration should be given to the fact that some studies show that extracellular nucleotides such as NAD^+ can also promote inflammation via the activation of **P2X7 receptors** in mouse macrophages and T cells [101].

The promise of NAD^+ boosters in the clinic

Adding more fuel

Given that low NAD^+ concentrations might exacerbate COVID-19 severity, boosting the levels of this metabolite in at-risk populations is one potential therapeutic strategy for mitigating severe disease. The most straightforward way to raise NAD^+ concentrations is to provide additional precursors to boost NAD^+ synthesis [2]. Canonically, NAD^+ cannot be taken up directly by the cell, but its precursors can [102,115]. The NAD^+ precursors nicotinic acid, NR, and NMN, all have putative transporters, high safety thresholds, and are orally bioavailable [2]. They do, however, vary in their pharmacokinetic profiles and seem to raise NAD^+ concentrations to different extents in different tissues [103], although this remains an area of active investigation.

Structurally, the closest molecule to NAD^+ is NMN, requiring only one enzymatic step to be converted to NAD^+. In a double blind, placebo-controlled trial, NMN was shown to increase insulin sensitivity in prediabetic women [28] (NCT03151239; Table 1). It is currently being studied in hospitalized COVID-19 patients with hypertension and metabolic syndrome for its effects on fatigue, duration of hospitalization, and viral load (NCT05175768; Table 1). Ongoing clinical trials with NMN are also investigating its effect on hypertension and postexercise muscle recovery (NCT04903210 and NCT04664361; Table 1).

NR, which is two enzymatic steps removed from NAD^+, is also being studied clinically. In one placebo-controlled trial, oral NR reduced circulating concentrations of inflammatory cytokines IL-2, IL-5, and IL-6 [104] (NCT02950441; Table 1). In another trial, oral NR reduced blood pressure and possibly aortic stiffness in healthy middle-aged and older adults [105] (NCT02921659; Table 1). At the time this review was written, over 30 active studies testing NR had been registered on clinicaltrials.gov, including many for COVID-19 risk factors such as vascular disease, hypertension, and heart failure (NCT04040959, NCT03821623, and NCT04528004; Table 1). At least four ongoing studies are directly testing NR in COVID-19 patients, some in patients with particular risk factors such as advanced age or acute kidney injury, with readouts such as rates of hospitalization, respiratory failure, and long-COVID symptoms (NCT04407390, NCT04818216, NCT04573153, and NCT04809974; Table 1).

Other approaches to raising NAD^+ concentrations

Another approach to raising NAD^+ concentrations in humans is to inhibit the activity of NAD^+-consuming enzymes. For instance, as PARP1 is a major consumer of NAD^+ in cells [42], PARP1 inhibitors such as olaparib have been extensively studied for the treatment of breast and ovarian cancer, given that PARP1 is synthetically lethal with *BRCA* mutations [106]. Regarding COVID-19, several PARP1 inhibitors, CVL218 and stenoparib, were recently shown to limit SARS-CoV-2 replication and proinflammatory cytokine production in human PBMCs and lung cells. One hypothesized mechanism of action is via inhibition of PARP1-dependent NAD^+ depletion [107,116].

Several inhibitors of other NAD^+-consuming enzymes are in preclinical development, including against CD38 [108,109], SARM1 [110], and ACMSD (which consumes a precursor of NAD^+ in the *de novo* pathway) [20]. In humans, treatment with luteolin, a naturally occurring CD38 inhibitor, reduced serum TNF and IL-6 concentrations [111].

The activation of NAD^+ biosynthetic enzymes is another strategy to raise NAD^+ concentrations; a small molecule NAMPT activator, SBI-797812, is currently in preclinical development for cardiovascular and metabolic disease [112]. Finally, a virally-encoded protein is a potential target that interferes with NAD^+ metabolism: the macrodomain of NSP3 can antagonize the antiviral activities of host PARPs, although the implications of this activity entail further investigation for SARS-CoV-2 [41,80–82]. A few inhibitors of NSP3 were recently identified in a chemical screen and were shown to exhibit antiviral properties against Chikungunya virus in human epithelial cells [113].

Concluding remarks

NAD^+ metabolism appears to be linked to infections of SARS-CoV-2 and other viruses via multiple lines of evidence, epidemiological and mechanistic. Further research is warranted to better understand its mechanistic role and utility as a target of intervention (see Outstanding questions). Clinical translation of basic scientific discoveries is difficult, and there are many examples of antiviral and anti-inflammatory molecules that have shown early promise but have not materialized. However, while human studies are still at an early stage, we are excited about the promise of interventions that modulate the concentrations of NAD^+ or the activity of NAD^+-consuming enzymes. Such interventions may soon have a place in our arsenal to fight current and future viral infections.

Outstanding questions

Why are NAD^+ tissue levels reduced during aging? Many hypotheses exist to date, but there is currently no consensus, especially as multiple mechanisms may be involved.

Are low NAD^+ concentrations associated with severe COVID-19? There is little data to date showing a direct association.

Can raising NAD^+ tissue concentrations reduce COVID-19 symptoms, severity, and mortality in the elderly and in the young?

Can NAD^+ boosters reduce COVID-19 severity after the onset of symptoms, or only prophylactically?

What might be the best approach for raising NAD^+ concentrations in the clinic: would this be NMN, NR, activators of NAD^+ biosynthesis, or inhibitors of enzymes that consume NAD^+?

What role does NAD^+ play in promoting SG-dependent antiviral mechanisms?

How does SARS-CoV-2 modulate NAD^+ and NSP3 to increase virulence?

Declaration of interests

D.A.S. is a founder, equity owner, advisor to, director of, board member of, consultant to, investor in and/or inventor on patents licensed to GlaxoSmithKline, Segterra, Animal Biosciences, AFAR, Cohbar, Galilei, Zymo Research, Immetas, EdenRoc Sciences and affiliates (Arc-Bio, Dovetail Genomics, Claret Bioscience, MetroBiotech, Astrea, Liberty Biosecurity and Delavie), Life Biosciences, and Levels Health. D.A.S. is an inventor on a patent application licensed to Elysium Health. More at https://sinclair.hms.harvard.edu/david-sinclairs-affiliations.

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