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# Viral infection as an NAD<sup>+</sup> battlefield

Coronavirus replication results in expenditure of nicotinamide adenine dinucleotide (NAD<sup>+</sup>), the central catalyst of cellular metabolism, in the innate response to infection. Repletion of NAD<sup>+</sup> levels has the potential to enhance antiviral responses.

## **Charles Brenner**

ince early 2020, the roughly 30-kb SARS-CoV-2 RNA genome has rewritten the rules for human life. Causing perhaps 250 million infections and 5 million deaths<sup>1</sup>, the polyprotein instructions on this positive RNA strand dictate assembly of active viral particles that do a remarkable job of spreading to others through aerosol droplets, causing life-threatening disease in a wide swath of infected individuals. The ongoing coronavirus pandemic has reorganized social and economic relationships globally. The potential of pets and wildlife to be currently incubating SARS-CoV-2 variants coupled with persistent vaccine hesitance and problems with vaccine distribution all limit confidence that any wave of infection is the last.

From their earliest replication intermediates, coronaviruses elicit an attack on cellular NAD+. Cellular NAD+ becomes a battlefield in which the innate immune system and the virus struggle for the upper hand. Based on preclinical work demonstrating transcriptional upregulation of NAD+-consuming enzymes and particular NAD<sup>+</sup> biosynthesis pathways<sup>2</sup>, early clinical data indicate that NAD+ repletion might constitute an antiviral treatment approach<sup>3</sup> that could be generally useful against infectious agents that activate the interferon system. The caveat—as with all approaches targeted to inflammation-is that inflammatory responses are themselves antiviral, although the severe cytokine storm associated with acute and chronic viral disease is considered a pathological overreaction.

Positive-strand RNA viruses, such as SARS-CoV-2, reveal a double-stranded RNA replication intermediate that binds to a pattern recognition receptor (PRR), the product of the retinoic acid-inducible gene I (*RIG-I*; also known as *DDX58*)<sup>4</sup>, and activates an antiviral immune response in naive cells. The RIG-I system leads to activation of interferon- $\beta$  (IFN $\beta$ ) transcription, followed by synthesis of IFN proteins that exit cells and bind to IFN receptors (IFNAR) in infected and neighboring cells. Interferon signalling



**Fig. 1** | **Deployment of NAD**<sup>+</sup> **in antiviral innate immunity.** Upon viral entry, pattern recognition receptors (PRRs) launch expression of inflammatory cytokines including IL6, IL10 and IFNβ. These molecules exit cells and act on the same or neighboring cells. Engagement of IFNβ with interferon receptors (IFNAR) leads to activation of the JAK-STAT pathway and interferon-stimulated gene (ISG) transcription. Among the ISGs, five members of the PARP superfamily are transcriptionally induced concomitant with expenditure of cellular NAD<sup>+</sup> and transcriptional upregulation of genes for nicotinamide (NAM) and nicotinamide riboside (NR) salvage. Repletion of cellular NAD<sup>+</sup> with NR is being tested as a means to boost the antiviral activities of the PARP superfamily members and has been found to be associated with calming the cytokine storm. NAM is undergoing clinical testing as an antiviral as well. ADPR, ADPribose; NAMPT, NAM phosphoribosyltransferase; NMN, NAM mononucleotide; NMRK, NR kinase.

proceeds through the JAK–STAT pathway to activate transcription of a set of interferon-stimulated genes (ISGs)<sup>5</sup> (Fig. 1).

As an innate response, the interferon system is, by definition, not educated by exposure to specific viral antigens and is highly similar in response to other molecular patterns, including those encoded by bacteria and unrelated viruses. Multiple ISG products have been shown to have potent antiviral activities. For example, higher circulating levels of 2'-5' oligoadenylate synthetase 1 (OAS1), a component of antiviral RNase L activity, are strongly associated with COVID-19 resistance<sup>6</sup>.

Assembly of a set of 71 genes that generate and respond to the NAD<sup>+</sup> metabolome<sup>7</sup> revealed that SARS-CoV-2 infection strongly induces transcription of

several genes with the potential to consume NAD<sup>+</sup> while also increasing expression of a subset of NAD<sup>+</sup> biosynthetic genes<sup>2</sup>. Among the NAD<sup>+</sup>-consuming enzymes, PARP7, PARP9, PARP10, PARP12 and PARP14 are named for their membership in the poly(ADPribose) polymerase superfamily. However, rather than polymerize ADPribose, these enzymes transfer single ADPribose units from NAD+ to polypeptide sidechains in order to modify protein functions<sup>8</sup>. Coronaviruses encode an enzymatic activity that removes ADPribose modifications<sup>9</sup>, suggesting that infected cells expend NAD<sup>+</sup> in antiviral ADPribosylating activities while the virus attempts to reverse these modifications. Indeed, coronavirus infection strikingly depletes cellular NAD+ levels, and boosting NAD+

pharmacologically or with NAD<sup>+</sup> precursors increases the enzymatic activity of antiviral PARP isozymes and inhibits coronavirus replication in vitro<sup>2</sup>.

Metabolism is the set of processes that allows us to convert everything we eat into everything we are and everything we do. The underlying chemistry of fuel oxidation, ATP generation, macromolecule synthesis and the generation and detoxification of reactive oxygen species involves electron flow in which the four NAD<sup>+</sup> coenzymes are the literal electrical conduits. Because cells struggle to maintain levels of NADH, NADP<sup>+</sup> or NADPH when NAD<sup>+</sup> is being churned by PARP activities, the data suggest that viral infection may generally impair host metabolic functions. It follows that if the induction of mono(ADPribosylating) enzymes is common to interferon system activation, chronic inflammation may be an indicator of a local or systemically stressed NAD<sup>+</sup> system, and a wide variety of bacterial and viral infections may similarly depress the NAD<sup>+</sup> system.

Model systems have found numerous examples in which tissue NAD<sup>+</sup> levels are depressed, for example in heart failure<sup>10</sup>, and peripheral<sup>11</sup> and central<sup>12</sup> neurodegeneration. These disturbances in NAD<sup>+</sup> are accompanied by transcriptional induction of nicotinamide (NAM) riboside (NR) kinase genes<sup>13</sup> as part of a homeostatic mechanism to restore NAD<sup>+</sup> metabolomes through the AMP kinase alarm system<sup>10</sup>. In response to coronavirus infection, NR kinase (NMRK) and NAM phosphoribosyltransferase (NAMPT) genes are upregulated, while NAD<sup>+</sup> biosynthetic capacity from other NAD<sup>+</sup> precursors is dampened<sup>2</sup>. These data suggested that NR or NAM as NAD<sup>+</sup> precursors or pharmacological activators of NAMPT might support the mono(ADPribosylating) activities of PARP in infection prevention or control.

Consistent with the view that viral infection leads to a bioenergetic crisis, blood from patients with severe COVID-19 contains lower levels of NAD<sup>+</sup> metabolites and higher levels of AMP<sup>14</sup>. In addition, dysregulation of NAD<sup>+</sup> metabolites and AMP in the blood of patients with severe COVID-19 correlates with high circulating levels of the inflammatory cytokines IL-6, IL-10, IL-8, M-CSF and IL- $1\alpha^{14}$ . Notably, inflammatory cytokine gene expression is downstream of PRR engagement and associated with antiviral defenses. However, persistent high-level expression is a sign of severe disease.

Small clinical studies have shown that oral supplementation with the NAD<sup>+</sup> precursor NR lowers circulation of inflammatory cytokines in healthy older adults15 and depresses expression of inflammatory cytokines in the peripheral blood mononuclear cells of people undergoing heart failure<sup>16</sup>. Following completion of a positive open-label phase 2 trial, a double-blinded phase 3 trial was completed, in which patients with mild-to-moderate COVID-19 were assigned to standard of care plus placebo (n = 75) or to standard of care plus a combination of four nutritional supplements, consisting of 3.7 g carnitine, 2.6 g *N*-acetylcysteine, 1 g NR and 12.4 g serine (n = 229), twice a day for two weeks3. Standard of care was either hydroxychloroquine<sup>17</sup> or favipiravir<sup>18</sup>, the repurposed malaria and flu drugs that do not have activity against SARS-CoV-2 in clinical trials.

Those on the supplement cocktail had a time to complete recovery of 5.7 days, compared with 9.2 days for those on placebo. This was significant at  $P < 2.0 \times 10^{-16}$  with a hazard ratio of 5.6 (95% confidence interval = 4.1–7.7). Consistent with a known activity of high-dose NR, those taking the supplement cocktail had reduced circulating levels of IL6, IL10, IFN $\gamma$ , CXCL10, CCL19, CX3CL1, CXCL11, IL-15RA, IL-17C, MCP-2 and TNF $\alpha^3$ .

Consistent with the view that any virus that engages the innate immune system to launch an interferon response attacks the NAD<sup>+</sup> system, zika virus infection has been shown to do just this in mouse brains<sup>19</sup>. Furthermore, NR protects cortex thickness, brain and body weight, and modestly improves survival of zika-infected mice when provided as an oral supplement<sup>19</sup>.

Mechanistic questions that remain to be resolved include the definition of the cellular or viral targets of the mono(ADPribosylating) PARP isozymes, and the dissection of how

age and pre-existing conditions modify inflammation, the NAD<sup>+</sup> system and antiviral defenses. Trials are being conducted to test whether NAM will protect against lymphopenia (NCT04910230) and the general disease course (NCT04751604) in COVID-19 and to determine whether NR will protect the kidneys (NCT04818216) and cognitive abilities (NCT04809974) of individuals with COVID-19 and those who have recovered from it, respectively. We also suggest prevention trials to determine whether fortification of the NAD<sup>+</sup> system may represent a relatively inexpensive way to protect exposed healthcare workers and housemates from viral infection. 

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#### **Competing interests**

C.B. developed intellectual property on nutritional and therapeutic uses of NR, which were commercialized by ChromaDex. C.B. owns ChromaDex stock and serves as their chief scientific advisor.

#### Peer review information

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