Nicotinamide pathways as the root cause of sepsis – an evolutionary perspective on macrophage energetic shifts

Melinda S. Suchard¹,² and Dana M. Savulescu¹

¹ Chemical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
² Centre for Vaccines and Immunology, National Institute for Communicable Diseases, a division of the National Health Laboratory Service, Johannesburg, South Africa

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Correspondence
M. S. Suchard, Chemical Pathology, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
Tel: +2711 386 6000
E-mail: melindas@nicd.ac.za

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Introduction

Sepsis, not to be confused with ‘septicaemia’ (bloodstream infection), is life-threatening organ dysfunction condition caused by the body’s own dysregulated response to infection [1]. Sepsis can be triggered by diverse pathogens and noninfectious insults. Why some people succumb to sepsis from triggers such as influenza or SARS-CoV-2 infection, while others have mild or asymptomatic infection, are unknown. Heightened susceptibility with old age is common with many infections [2,3], as is the association with comorbidities often described as immunosuppressive, including type 2 diabetes [3–7]. While many eyes focus on pathogen itself, a closer look at host immune and metabolic responses to diverse pathogens is critical.

Avenues of investigation from both ageing research and immunology are converging attention on a pathway, which connects immune responses with ageing [8–13]. Nicotinamide adenine dinucleotide (NAD) in its oxidized (NAD⁺) and reduced (NADH) forms plays an essential role in energy metabolism in every eukaryotic cell [14,15]. Ageing research has shown that NAD metabolism is crucial in determining ‘healthy ageing’ [16]. Declining NAD⁺ levels and reduced NAD⁺ : NADH ratios are associated with age-related declines in cellular functions [17]. NAD-degrading enzymes including poly(ADP-ribose) polymerases (PARPs), sirtuins and CD38 have similarly been linked with ageing. Sirtuins are NAD⁺-dependent proteins involved in longevity
associated with caloric restriction [18]. Similarly, advances in immunology have shown that de novo production of nicotinamide, a precursor of NAD⁺, is a central pathway upregulated in many infectious diseases [8,19]. In the macrophage, a central mediator of immune responses, de novo NAD⁺ synthesis was required to maintain an ‘anti-inflammatory homeostatic state with robust phagocytic capacity’ [8].

We propose that sepsis due to SARS-CoV-2 or other viral or bacterial infections is caused by pathogens interfering with host NAD metabolism. Elderly individuals, or those with pre-existing diseases, may already have certain aberrations in their NAD metabolic pathways that predispose them to more severe disease [20]. We will review recent understanding of metabolic shifts in cells of the immune system, particularly macrophages, and link macrophage metabolic shifts to intracellular NAD concentration as a focal point. We use an evolutionary perspective to motivate that alterations in NAD metabolism may be causal rather than merely correlated with infectious disease.

### Nicotinamide and macrophage phenotypes

In humans, nicotinamide is acquired from dietary sources as vitamin B3 and subsequently recycled through existing nicotinamide pools. Alternatively, nicotinamide may be synthesized de novo from the amino acid tryptophan. The rate-limiting enzyme for de novo nicotinamide synthesis is indoleamine 2,3-dioxygenase (IDO), a haem-containing intracellular enzyme found predominantly in cells of the macrophage/monocyte lineage.

Macrophages have long been described as able to become activated in one of two ways, simplistically thought of as ‘pro-inflammatory’ and ‘anti-inflammatory’ or termed by ‘M1’ and ‘M2’ nomenclature. Technicalities and disputes regarding nomenclature abound [21], but the principle holds that macrophages have at least two divergent ways of becoming activated, rather than the stereotypical pro-inflammatory textbook version. M1 macrophages secrete pro-inflammatory cytokines such as TNF alpha and interleukin 1-β, while M2 macrophages secrete cytokines such as interleukin-10, which have immune suppressive functions and play a role in wound healing.

Key enzyme activities differentiate between M1 and M2 macrophages. From arginine as a substrate, M1 macrophages use nitric oxide synthase to make nitric oxide, while M2 macrophages use arginase to catabolize arginine to urea [22]. Most strikingly, M2 macrophages express IDO, which diverts tryptophan to de novo synthesis of nicotinamide rather than towards serotonin synthesis [23]. IDO-mediated tryptophan catabolism in turn depresses T-cell proliferation and stimulates regulatory T cells, which are cells with immune suppressive function [24].

Counterintuitively, IDO is induced by pro-inflammatory cytokines such as interferon-γ [25]. The IDO-catalysed de novo nicotinamide synthesis pathway is therefore upregulated during diverse infectious conditions as a response to pro-inflammatory cytokines [26]. We can infer that the IDO pathway may be part of a negative feedback loop to dampen inflammation.

### Immunometabolism in macrophages

Recent work has led to the burgeoning field of immunometabolism [19,27,28]. Not only do M1 and M2 macrophages differ in the cytokines produced and enzymes activated, but also they differ in their glucose metabolism [29,30]. Immune cells, including macrophages, lymphocytes and neutrophils, can switch between the ‘resting’ state, during which the cell undergoes glycolysis, Krebs cycle and oxidative phosphorylation, and an activated state in which aerobic glycolysis (also known as Warburg-like metabolism) occurs [29,30]. During Warburg-like metabolism, glycolysis (aerobic glycolysis) is completed without further completion of a traditional Krebs cycle and electron transport chain. Warburg-like metabolism, originally identified as a hallmark of cancer cells, is often associated with cell division and cell activation [30–32]. When infected with pathogens, macrophages shift their metabolism to a Warburg-like metabolism, with potential pathogen-specific variations [19,33]; for example, Legionella pneumophila, Chlamydia trachomatis and Mycobacterium tuberculosis may impact metabolic pathways at different points, all upregulating aerobic glycolysis [19]. In T and B lymphocytes, shifting to Warburg-like metabolism is associated with lymphocyte proliferation [34].

### Evolutionary perspective on immunometabolism

To elucidate how macrophage or lymphocyte metabolic shifts correlate with infection by ‘foreign’ microorganisms, an evolutionary perspective may be of value. All eukaryotic cells require NAD and NADH for metabolism [15,35]. This commonality stems from the origin itself of eukaryotic life, when two prokaryotes (an α-proteobacterium and an archaeon) fused, after which the α-proteobacterium became the mitochondrion [36].
Mitochondrial function within the eukaryotic cell largely revolves around a hydrogen-dependent symbiosis, mediated through NAD and NADH [37]. Mitochondria control not only the life but also the death of the cell, including initiation of programmed cell death pathways [38]. Viewed in this light, the switch to aerobic glycolysis (Warburg-like metabolism) during times of immune activation is indicative of a change in mitochondrial function, almost as if the cell reverts to a more prokaryotic form of metabolism, becoming temporarily independent of its need for symbiotic mitochondrial metabolism.

More recently in evolutionary terms, focusing our attention on human reproductively fitness and immune tolerance may illuminate triggers for IDO production. In addition to macrophages, IDO is expressed in placental tissue and is essential for mammalian immune tolerance of an allogeneic, but not syngeneic fetus [39]. During sperm–ovum fusion, the ovum actively targets and destroys male mitochondria, a process known as mitophagy or allotrophy [40,41]. The mechanism of sensing which mitochondria are paternal remains unelucidated. Mitochondrial contents are reminiscent of their bacterial origins, differing from nuclear or cytoplasmic contents by the presence of unmethylated CpG motifs in mitochondrial DNA [42], possession of unique lipids including cardiolipin [43] and synthesis of double-stranded RNA intermediates [44], which can trigger innate immune responses if released into the cytoplasm or circulation [45]. Mitochondria themselves harbour receptors, such as the mitochondrial antivirally signalling protein (MAVS), that can be triggered by double-stranded RNA [46]. It is plausible that mitochondrial sensing of double-stranded RNA, or other mitochondrial patterns, is a mechanism that has been conserved through evolution, stemming from similar functions during mitophagy at conception. Mitochondrial switching between oxidative phosphorylation and Warburg-like metabolism may be the response of the mitochondria to sensing foreign mitochondrial patterns within the cell, both during reproduction and during infection. We hypothesize that mitochondrial sensing of foreign double-stranded RNA or other mitochondrial identifiers increases the cellular requirement for NAD⁻⁺, thereby prompting increased tryptophan conversion to nicotinamide via IDO activation. Such a requirement for increased de novo nicotinamide production could explain the requirement for IDO activity in materno-fetal tolerance at the placenta. Intersection of NAD pathways with mitochondrial metabolism points in the direction of mitochondrial metabolic shifts correlating with altered cellular NAD requirements.

**NAD requirements of the macrophage may prompt metabolic shifts**

In M2 macrophages, a fully functional Krebs cycle and oxidative phosphorylation within the mitochondrion corresponds to increased IDO activity within the cytoplasm (Fig. 1). In classically activated macrophages, however, a shift that can be triggered during diverse infections, mitochondria undergo only limited function [19,33]. Sensing of double-stranded RNA is known to trigger conserved mitochondrial response pathways [47–49]. We hypothesize that mitochondrial response to double-stranded RNA precipitates an acute cellular NAD insufficiency. A detectable increase in IDO activity would be a measure of the body’s attempt to compensate for insufficient nicotinamide. Elevated IDO activity for de novo nicotinamide synthesis would be termed an initial M2 shift. If de novo nicotinamide synthesis failed to meet cellular NAD requirements, the macrophage may shift to classical activation, speculatively due to a lower NAD requirement.

**A closer look at pathways intersecting with NAD**

Cameron and colleagues showed in vitro that during Warburg-like metabolism, reactive oxygen species were upregulated, DNA damage occurred and PARP enzymes were upregulated, depleting NAD⁺. M1 macrophage activation occurred simultaneously with NAD⁺ depletion and upregulation of the NAD salvage enzyme, nicotinamide phosphoribosyltransferase (NAMPT) [50]. The authors attributed early depletion of NAD⁺ to PARP activity, but noted that sirtuins and CD38 may also have been involved with NAD⁺ depletion. Their work illustrated that NAD depletion was a key feature of M1 activation, but multiple hypotheses may apply as to the initial cause of NAD⁺ depletion and PARP activation [50].

Zhang et al investigated low-dose compared with high-dose endotoxin responses in vitro. They showed that low-dose endotoxin triggered upregulation of NAMPT, resulting in NAD salvage from nicotinamide, while high-dose endotoxin caused a switch to de novo nicotinamide synthesis from tryptophan via IDO [51]. NAD synthesis (via NAMPT or IDO) affected nuclear NAD⁺ pools, which could affect transcription of key inflammatory genes.

NAD⁺ is released from cells during early inflammation and functions as an extracellular signalling molecule, resulting in death of naive CD4⁺ and CD8⁺ T cells while relatively sparing activated and memory cells [52]. The ectoenzyme CD38, an activation marker on T and B
lymphocytes, functions as an NADase, regulating extracellular concentrations of NAD$^+$ [52]. CD38 can also be upregulated on airway smooth muscle cells in response to inflammatory cytokines [53]; thus, the NADase activity of CD38 is not limited to immune cells [54]. NAD$^+$ also plays a key role in autophagy through partnering with sirtuins, which are NAD-dependent deacetylases [55]. Autophagy relates to degradation of intracellular organelles, often in response to nutrient stresses [55]. Sirtuins regulate autophagy [55], as well as circadian rhythms in the cell [56]. The intersection of mitochondrial, cytoplasmic, nuclear and extracellular NAD pools is critical for key cellular functions including DNA repair (via PARP enzymes), autophagy (via sirtuins) and activation or suppression of surrounding lymphocytes (via CD38) [57].

**Interaction of infectious organisms with de novo nicotinamide synthesis**

Interestingly, IDO activity is elevated in human sepsis and severe inflammatory response syndrome (SIRS), with higher values predicting mortality [58]. Patients with sepsis of diverse origins have increased kynurenine and decreased tryptophan plasma concentrations [59–61]. Kynurenine/tryptophan ratios are higher in patients with severe sepsis than in those with mild sepsis, and were inversely related to microvascular reactivity [59]. Kynurenine has blood vessel-relaxing properties [62], and it has been suggested that IDO activity links together immune dysregulation and loss of microvascular reactivity in sepsis [59].

Many bacteria use NAD and NADH in their own metabolism. As prokaryotes, bacteria have their own unique pathways of NAD synthesis. Commensal and pathogenic flora form a complex cycle of NAD synthesis and consumption, which may regulate the pathogen–host balance [19,63]. As an example, tuberculosis-necrotizing toxin, secreted by Mycobacterium tuberculosis, with homologues in many bacterial and fungal pathogens, hydrolyses NAD$^+$ and results in cellular death by necroptosis [64]. Mycobacterium tuberculosis can also synthesize niacin [65], leading to postulates that latent Mycobacterium tuberculosis has beneficial effects for the human host by increasing nicotinamide availability [63,66].

Viruses cannot synthesize their own NAD or NADH but usurp host metabolism to engage with the
host NAD pathways; for example, acute and chronic viral infections affect IDO activity and nicotinamide pathways [67–69]. Cytomegalovirus, influenza virus, herpesviruses 1 and 2, hepatitis B, hepatitis C and human immunodeficiency virus (HIV) interact with the IDO pathway [67–69]. IDO activity is elevated during HIV infection, resulting in raised kynurenine/tryptophan ratios. IDO activity represents an independent marker of disease progression to acquired immunodeficiency syndrome (AIDS), whereas antiretrovirals decrease the kynurenine/tryptophan ratio [69]. Notably, acute viral infection with influenza virus induces IDO activity [70]. Importantly, in the Human Influenza INSIGHT FLU 003 Plus study, increased IDO activity was associated with poor clinical outcome (death, transfer to intensive care or requiring mechanical ventilation) [71].

Patients with severe disease from SARS-CoV-2 infection (coronavirus disease 2019 or COVID-19) often have lymphopenia together with high white cell counts, suggesting involvement of myeloid cells in disease pathogenesis [72]. Lung COVID-19 has been ascribed to hyperinflammatory syndrome or cytokine release occurring in the later phases of the illness [72], with some descriptions akin to macrophage activation syndrome [73,74]. The replication of SARS-CoV-2, similar to many other viruses [75], comprises a double-stranded RNA step, which may be sensed by mitochondrial antiviral mechanisms [76]. SARS-CoV-2 has been shown experimentally to upregulate PARP enzymes, which degrade NAD⁺ [77]. Higher titres of SARS-CoV-2 differentially regulated various PARP enzymes and downregulated quinolinic acid phosphoribosyltransferase, an enzyme required for de novo nicotinamide synthesis from tryptophan, while upregulating NAMPT, which utilizes nicotinamide to synthesize NAD⁺ [77]. In a transcriptomics analysis, SARS-CoV-2 downregulated nuclear-encoded mitochondrial genes coding for complex 1 of the mitochondrial electron transport chain [78]. Thus, evidence exists to suggest that SARS-CoV-2 interacts with host NAD⁺ metabolism. Intriguingly, loss of smell and taste, symptoms associated with COVID-19, was recognized in the 1930s as pellagra symptoms, amenable to niacin treatment [79].

The link between infection, NAD⁺ consumption and metabolic shifts in macrophages

Shifts in macrophage metabolism during infection are only partially understood, and opposing explanations may fit the observations described. Our preferred hypothesis is that viral infection initially triggers increased consumption of NAD⁺ or decreased availability of NAD⁺ for the human host. The IDO pathway is then upregulated to replenish NAD⁺ supplies (an initial M2 shift), and when capacity of the de novo pathway is exceeded, a relative deficiency of NAD⁺ may force a shift to M1 phenotype. Alternative explanations have not been excluded; for example, infection may initially trigger a shift to an M1 phenotype, speculatively leading to decreased NAD⁺ availability, after which IDO is upregulated in order to meet the need for de novo synthesis, which would be seen as a secondary M2 shift. Further work will elucidate these intricacies, but the understanding that pathogens induce shifts in human metabolism leads to practical host-directed interventions. Kynurenine biosynthesis via IDO upregulation is a signature of infection with a wide range of pathogens [68]. Increased kynurenine biosynthesis implies that diverse microbes trigger a convergent host response of increased de novo nicotinamide synthesis, likely secondary to an increased NAD⁺ requirement. Interventions targeted at the IDO-catalysed pathway may therefore ameliorate severe illness, despite diverse aetiologies.

Novel therapies for sepsis

If the primary pathology is competition for depleted NAD⁺ stores, the most rational intervention would be to increase NAD⁺ supply. The leading candidate for investigation for treatment of sepsis, including COVID-19, should therefore be nicotinamide (Vitamin B3) and related compounds, in agreement with other authors [20,77,80–85]. Indeed, in animal models, vitamin B3 ameliorates polymicrobial sepsis [86], lung ischaemia–reperfusion injury [87] or experimentally induced lung fibrosis [88]. In animals, nicotinamide mononucleotide preserves mitochondrial function and promotes survival from haemorrhagic shock [89]. Importantly, in a mouse model of SARS-CoV-2 infection, nicotinamide administration reduced inflammatory cell aggregates, emboli and cell death [90]. We will focus further on the experience with administration of vitamin B3 and related compounds to humans.

Nicotinamide has been used for a multitude of clinical indications, including lung diseases, as outlined below. The stalwart of antituberculosis therapy, isoniazid, was initially developed as a nicotinamide analogue, but showed superior performance compared with nicotinamide for clinical outcome in Tuberculosis [91]. Indeed, pyrazinamide – another antituberculosis agent – has downstream metabolites, which convert nicotinamide to NAD⁺ [10]. Thus, at least some of the efficacy of isoniazid and pyrazinamide for treatment of sepsis caused by...
tuberculosis may be due to interactions of the drugs with NAD synthesis and salvage [63].

There is extensive prior experience with nicotinamide in human trials for conditions other than infections [92–98]. Nicotinamide forms part of certain cancer treatment regimens [93,94]. The recommended daily dose of nicotinamide is 14–16 mg, and nutritional supplementation dosages are usually below 35 mg day−1. The upper recommended limit for adults is, however, 900 mg day−1 [99], and doses used for treatment of malignancy are approximately 3 g day−1. Impaired oxidative burst activity of neutrophils in type 2 diabetics was improved by nicotinamide supplementation at 50 mg kg−1 day−1 [100]. Dosage of nicotinamide for clinical trials should thus span physiological and higher ranges for thorough assessment of clinical impact.

In addition to experience with nicotinamide, three related compounds interact at various points in NAD metabolism and are options for human therapeutic trials for sepsis. Niacin is a clinically licensed therapy for hypercholesterolaemia; however, its use may be limited by side effects such as flushing. Ageing experts recommend an alternate product, nicotinamide mononucleotide, as their drug of choice for prevention of age-related declines, with multiple examples in animal models [101,102] and safety data in humans [103]. Nicotinamide mononucleotide may have superior properties to nicotinamide in terms of its activity and side effect profile [102]. Importantly, dramatic clinical improvement was reported in a case series of nine severe COVID-19 patients treated with a nicotinamide mononucleotide cocktail (nicotinamide mononucleotide, betaine, sodium chloride and zinc sulfate) [85]. A third compound, nicotinamide riboside, has shown safety in human trials and ability to raise whole blood NAD+ levels [104]. Some have suggested that for treatment of COVID-19, administration of nicotinamide-related compounds should be accompanied by an inhibitor of PARP enzymes, which degrade NAD+ [83].

**Conclusion**

In summary, we advocate adjusting our understanding of pathogenesis of infectious illness away from a microbe-oriented view, such that the microbe is seen as ‘causing’ the disease, towards a host NAD–metabolism-oriented view, where the microbe is seen as triggering an evolutionarily conserved response that shifts NAD metabolism. In certain contexts, such as during reproduction, shifts in NAD metabolism may be beneficial for the organism. In the context of disease, such shifts are associated with adverse consequences. Understanding interactions of various bacterial and viral pathogens with the NAD pathway will guide us further. While biochemical studies will ultimately yield mechanistic explanations, a more direct approach is to investigate vitamin B3-related compounds in human trials for sepsis.

The de novo nicotinamide synthesis pathway, catalysed by IDO, poses a therapeutically malleable pathway integrally linked to host mitochondrial metabolism and to immune tolerance. Understanding the relationship between pathogenic infection and macrophage metabolism, and identifying how to diagnose and direct flux through the de novo nicotinamide synthesis pathway, should lead towards host-directed therapy for sepsis. Host–microbe competition and interaction for limited intracellular NAD+ supplies are the lens through which we should view mitochondrial metabolic shifts within cells of the immune system.

In conclusion, we strongly suggest that vitamin B3 be investigated as a therapy for sepsis, including that caused by COVID-19, ideally as a single agent at high dose rather than within a multivitamin, which will not allow accumulation of efficacy data. If nicotinamide is found to be ineffective, related compounds including isoniazid, niacin, nicotinamide riboside and nicotinamide mononucleotide are alternate prospects that impact NAD pathways at different entry points of the NAD cycle and warrant further investigation.

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**Conflict of interest**

The authors declare no conflict of interest.

**Author contributions**

MSS conceptualized and wrote the piece. DMS edited the manuscript and gave critical comment.

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