IMMUNOMETABOLISM

# NAD-biosynthetic pathways regulate innate immunity

Recent studies indicate that macrophages utilize NAD⁺-biosynthetic pathways to control inflammation and cell survival during the immune response and aging.

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Nicotinamide adenine dinucleotide (NAD⁺) biology has garnered much attention over the past few years. A decrease in NAD⁺ abundance has been linked to many age-related diseases and is associated with changes in metabolism that alter cellular function[1]. Activation of macrophages is accompanied by distinct metabolic changes that are necessary to drive their pro-inflammatory state[2]. Therefore, many strategies for boosting NAD⁺ levels have piqued interest as a means by which to diminish inflammation and pathologies incurred during normal aging[3]. Minhas et al.[4] and Cameron et al.[5] have now demonstrated the necessity of de novo NAD⁺ synthesis in macrophage-driven inflammation during aging, as well as the importance of the NAD⁺ salvage pathway for maintenance of the pro-inflammatory state.

NAD⁺ has multiple well-described functions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) converts NAD⁺ into NADH to catalyze the simultaneous phosphorylation and oxidation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate, a key step in glycolysis. Furthermore, NAD⁺ is reduced to NADH to drive multiple key steps in the mitochondrial tricarboxylic acid (TCA) cycle. Subsequently, NADH is oxidized back to NAD⁺ by complex I of the mitochondrial electron-transport chain. In contrast to the cellular functions that cycle NAD⁺ and NADH, others consume NAD⁺. Poly(ADP-ribose) polymerases (PARPs), which promote DNA-damage repair and maintenance of genomic integrity, the cyclic ADP-ribose synthase CD38, a critical mediator of immune cell activation and adhesion, and sirtuins, which possess deacylase activity as part of stress responses, all consume NAD⁺. Interestingly, aging is associated with increased PARP activity due to increases in DNA damage as well as heightened expression of CD38 in inflammatory cells; this leads to a decrease in NAD⁺ abundance that is accompanied by diminished sirtuin activity[6]. Dietary supplementation with nicotinamide (NAM), nicotinamide mononucleotide (NMN) and nicotinamide riboside is able to boost NAD⁺ levels and restore sirtuin activity in aged mice[3].

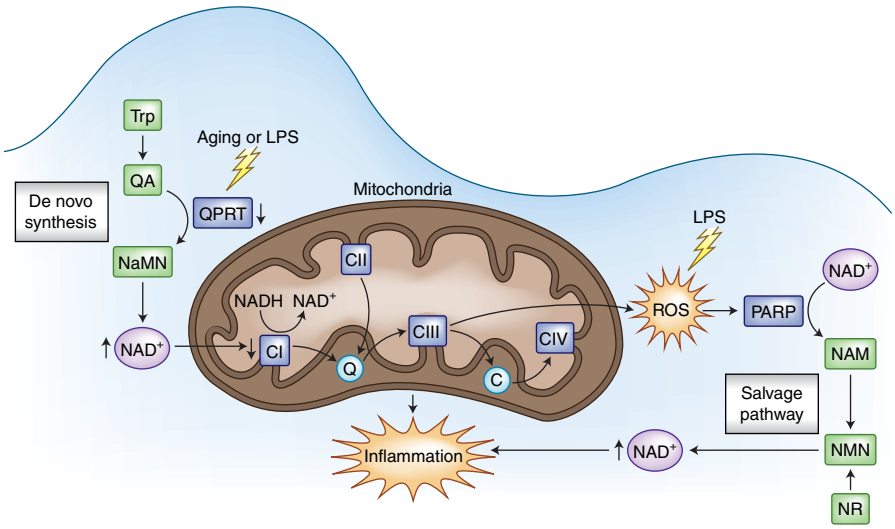


**Fig. 1 | NAD⁺ pathways that control macrophage dependent inflammation.** NAD⁺ is synthesized from tryptophan (Trp) through the de novo synthesis pathway (via quinolinic acid (QA) and nicotinic acid mononucleotide (NaMN)) or is recycled from NAM through the salvage pathway (via nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN)). During aging or in response to LPS, the abundance of the de novo–synthesis protein QPRT drops, which leads to a diminished NAD⁺ pool. The decreases in NAD⁺ levels result in decreased electron-transport chain complex I (CI) activity and increased inflammation. Stimulation with LPS also stimulates the generation of ROS from complex III (CIII) of the electron-transport chain. This increase in ROS results in DNA damage and the subsequent stimulation of PARP enzymes, which consume NAD⁺ to repair damaged DNA and maintain genomic integrity. The NAD⁺ salvage pathway replenishes the NAD⁺ pool, which allows cell survival and initiation of the pro-inflammatory response.

The intracellular NAD⁺ concentration is the balance between NAD⁺-consumption pathways and NAD⁺ synthesis via three distinct pathways: the de novo biosynthetic pathway, the Preiss–Handler pathway, and the salvage pathway. De novo NAD⁺ biosynthesis begins with metabolism of the amino acid tryptophan to kynurenine by indoleamine-2,3-dioxygenase. Kynurenine is then metabolized through the kynurenine pathway to quinolinic acid, which is converted by quinolate phosphoribosyltransferase (QPRT) to nicotinic acid mononucleotide. The Preiss–Handler pathway metabolizes kynurenine pathway–derived nicotinic acid mononucleotide or diet-derived nicotinic acid into NAD⁺. In the salvage pathway, nicotinamide phosphoribosyltransferase (NAMPT) metabolizes NAM to NMN, which is then converted into NAD⁺ (ref. [7]).

Immune cells upregulate NAMPT in response to inflammatory stimuli, which suggests that the $NAD^+$ salvage pathway is the main means by which they maintain $NAD^+$ levels during activation[8]. Moreover, a published study has indicated that de novo $NAD^+$ synthesis is thought to occur mainly in the liver, with hepatocytes releasing NAM into the bloodstream for other tissues to take up and metabolize[9]. However, Minhas et al. demonstrate that macrophages perform de novo $NAD^+$ synthesis both in vitro and in vivo under resting conditions[4]. Genetic or pharmacological inhibition of QPRT elevates the inflammation-associated TCA-cycle metabolite succinate, decreases mitochondrial respiration due to diminished complex I activity and increases mitochondria-generated reactive oxygen species (ROS). Such observations are consistent with the literature, which has shown that inflammatory macrophages induced via lipopolysaccharide (LPS) exhibit a distinct metabolic profile characterized by increases in succinate as well as increased mitochondrial production of ROS[10]. Inhibition of QPRT increases inflammatory markers in mouse and human macrophages without LPS stimulation. In the absence of de novo $NAD^+$ synthesis, the activity of Sirt3, a $NAD^+$-dependent mitochondrial sirtuin, is diminished despite no change in the abundance of Sirt3 protein[4].

Minhas et al. also report that despite an increase in metabolites between kynurenine and quinolinic acid in the de novo synthesis pathway, $NAD^+$ levels decrease in response to LPS and aging[4] (Fig. 1). That finding is due to a combination of increased consumption of $NAD^+$ by PARPs and a decrease in QPRT expression. In the absence of QPRT activity, the induction of pro-inflammatory cytokines via LPS is elevated. Supplementation with NMN or overexpression of QPRT in human macrophages restores mitochondrial respiration and Sirt3 activity and normalizes TCA-cycle intermediates to homeostatic, anti-inflammatory levels during LPS treatment. Thus, macrophages activated with LPS exhibit decreased $NAD^+$ due to depletion of the $NAD^+$ pool by $NAD^+$-consuming enzymes and inhibition of de novo $NAD^+$ synthesis, which leads to decreased Sirt3 activity and a metabolic profile consistent with a pro-inflammatory phenotype. Strengthening the links among inflammation, $NAD^+$ metabolism and aging, overexpression of QPRT in macrophages from donors 65 years of age or older is sufficient to return their inflammatory and metabolic profile to one consistent with that of human macrophages from donors under 35 years of age. Together, the findings of Minhas et al. identify a role for the de novo synthesis of $NAD^+$ in maintaining an anti-inflammatory phenotype in resting macrophages[4].

Cameron et al. explore the involvement of the $NAD^+$ salvage pathway in controlling the pro-inflammatory state of macrophages[5]. Strikingly, they report that a drop in $NAD^+$ levels occurs within an hour of the stimulation of macrophages with LPS. Concurrently, an increase in the transcription of genes encoding proteins in the $NAD^+$ salvage pathway occurs. Hence, the $NAD^+$ salvage pathway is upregulated to prevent $NAD^+$ levels from dropping to concentrations that would compromise the survival and function of inflammatory macrophages. Mechanistically, LPS increases the mitochondrial complex III generation of ROS, which results in DNA damage. This damage stimulates PARP activity, which consumes $NAD^+$ and depletes the $NAD^+$ pool in the cell. Consequently, macrophages stimulate the NAMPT salvage pathway in order to sustain $NAD^+$ levels (Fig. 1). Accordingly, inhibition of NAMPT causes a decrease in $NAD^+$ to levels that result in a decrease in GAPDH activity and diminishes the induction, by LPS, of genes encoding inflammatory molecules in vitro. Inhibition of NAMPT also reduces disease severity in an LPS-induced model of sepsis. Supplementation in vitro with the $NAD^+$ precursor NMN 'rescues' the effects of the inhibition of NAMPT on GAPDH activity and the production of pro-inflammatory cytokines. Interestingly, dimethyl fumarate, a derivative of the TCA-cycle intermediate fumarate, is used to treat multiple sclerosis and psoriasis. Dimethyl fumarate is a strong electrophile that covalently modifies GADPH and decreases its activity to diminish inflammation. It is not clear why decreasing the activity of GAPDH, which maintains the upstream functions of glycolysis (such as the pentose-phosphate and hexosamine pathways) but diminishes de novo serine synthesis as well as the production of ATP and pyruvate, decreases inflammation[11].

An interesting aspect of the study by Cameron et al. is the finding that mitochondrial complex III production of ROS not only triggers the decrease in $NAD^+$ levels during stimulation with LPS but also is necessary for the induction of pro-inflammatory cytokines[5]. A published study has suggested that reverse electron transport (RET) from mitochondrial complex II to mitochondrial complex I generates the ROS production necessary for induction of the pro-inflammatory cytokine IL-1β by LPS[10]. However, Cameron et al. demonstrate that inhibition of complex III, which would promote RET, decreases the induction of IL-1β by LPS, rather than increasing it[5]. Moreover, Minhas et al. demonstrate that diminished activity of complex I due to decreases in de novo $NAD^+$ synthesis increases inflammation[4]. Such findings are consistent with the published observation that genetic deletion of the complex I subunit NDUFS4, which causes diminished complex I activity, results in hyper-inflammation[12]. Furthermore, a published finding has demonstrated that overexpression of alternative oxidase (AOX) decreases the induction of inflammation by LPS in vitro and in vivo[10]. AOX, like mitochondrial complex III, takes electrons from ubiquinol but does not generate ROS[10]. It has been suggested that AOX prevents the generation of ROS via mitochondrial complex I RET. However, it is likely that AOX prevents the generation of ROS by complex III and thereby decreases inflammation. Collectively, these studies provide an impetus to reexamine the role of complex I–driven RET production of ROS in the induction of inflammation by LPS.

In the study by Minhas et al., reducing the de novo synthesis of $NAD^+$ decreases inflammation[4], and in the study by Cameron et al., synthesis of $NAD^+$ by the salvage pathway drives a fulminant macrophage inflammatory response to LPS[5]. A possible explanation for this is that de novo pathways affect the generation of $NAD^+$ to support the activity of mitochondrial complex I and thus maintain respiratory function. In contrast, the salvage pathway is needed to sustain glycolysis through the maintenance of GAPDH activity. An emerging theme in metabolism is renewed recognition of the importance of metabolic compartmentalization in the control of biological function.

Macrophage dysregulation has been linked to many age-related pathologies. The findings of Minhas et al.[4] and Cameron et al.[5] should prompt investigators to assess macrophage activation in pathological settings in which $NAD^+$ levels are dysregulated. We are tempted to speculate that perhaps the pleiotropic effects of NAD-boosting agents might be due to their ability to modulate inflammation, similar to other drugs such as aspirin, metformin and statins. ❐

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## References


1. Katsyuba, E. & Auwerx, J. *EMBO J.* **36**, 2670–2683 (2017).
2. Murphy, M. P. & O'Neill, L. A. J. *Cell* **174**, 780–784 (2018).
3. Yoshino, J., Mills, K. F., Yoon, M. J. & Imai, S. *Cell Metab.* **14**, 528–536 (2011).
4. Minhas, P. S. et al. *Nat. Immunol.* **20**, 50–63 (2019).
5. Cameron et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-019-0336-y (2019).
6. Camacho-Pereira, J. et al. *Cell Metab.* **23**, 1127–1139 (2016).
7. Verdin, E. *Science* **350**, 1208–1213 (2015).
8. Grahnert, A. et al. *Innate Immun.* **17**, 212–233 (2011).
9. Liu, L. et al. *Cell Metab.* **27**, 1067–1080.e5 (2018).
10. Mills, E. L. et al. *Cell* **167**, 457–470.e13 (2016).
11. Kornberg, M. D. et al. *Science* **360**, 449–453 (2018).
12. Jin, Z., Wei, W., Yang, M., Du, Y. & Wan, Y. *Cell Metab.* **20**, 483–498 (2014).


## Competing interests

The authors declare no competing interests.