● **INVITED REVIEW**

Kynurenine pathway metabolism and neuroinflammatory disease

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Abstract

Immune-mediated activation of tryptophan (TRYP) catabolism *via* the kynurenine pathway (KP) is a consistent finding in all inflammatory disorders. Several studies by our group and others have examined the neurotoxic potential of neuroreactive TRYP metabolites, including quinolinic acid (QUIN) in neuroinflammatory neurological disorders, including Alzheimer's disease (AD), multiple sclerosis, amylotropic lateral sclerosis (ALS), and AIDS related dementia complex (ADC). Our current work aims to determine whether there is any benefit to the affected individuals in enhancing the catabolism of TRYP *via* the KP during an immune response. Under physiological conditions, QUIN is metabolized to the essential pyridine nucleotide, nicotinamide adenine dinucleotide (NAD^+), which represents an important metabolic cofactor and electron transporter. NAD^+ also serves as a substrate for the DNA 'nick sensor' and putative nuclear repair enzyme, poly(ADP-ribose) polymerase (PARP). Free radical initiated DNA damage, PARP activation and NAD^+ depletion may contribute to brain dysfunction and cell death in neuroinflammatory disease.

Key Words: kynurenine pathway; tryptophan; NAD^+; PARP; inflammation

The Kynurenine Pathway in Neuroinflammatory Disorders

TRYP is the least abundant amino acid in mammalian organisms, and accounts for only 1–1.5% of the total protein amino acid content. However, it was not until the late 1970s and 1980s that KP generated considerable interest among neuroscientists, since it was discovered that QUIN and kynurenine acid (KYNA), (two metabolites of the KP) exhibited significant and opposing actions on neuronal cells. QUIN is a selective agonist at the N-methyl D-aspartate (NMDA) site of the excitatory NMDA (glutamate subtype) receptor, while KYNA is an antagonist at both the NMDA and glycine site of this ionotropic receptor (Guillemin, 2012). Activation of the NMDA receptor has been shown to permeate cells to Ca^{2+}, Na^+ and K^+ ions. Increased intracellular Ca^{2+} influx has been shown to activate several secondary messenger signalling pathways leading to synaptic alterations. Moreover, increased intracellular Ca^{2+} influx due to excessive NMDA receptor activation can induce excitotoxicity and neuronal cell death in several neurodegenerative diseases (Braidy et al., 2010).

In humans, CNS QUIN levels are increased in several neurological disorders including AD, depression, epilepsy, autism, schizophrenia, and patients are more susceptible to suicide risk (Brundin et al., 2016). This has led to the hypothesis that the increase in CNS levels of QUIN is pivotal to the pathogenesis of these disorders through its mode of action at the NMDA receptor. QUIN may also induce toxicity to brain cells *via* exogenous free radical production. On the contrary, reduced levels of KYNA have been reported in neurological disorders such as Huntington's disease (HD), and AD. It has been suggested that the development of inflammatory mediated neuropathology is correlated to changes in the ratio of KYNA to QUIN rather than QUIN levels alone.

Given the extent that the KP influences neuronal function, identifying the site of TRYP metabolite production during CNS inflammation is beneficial to gain a sound understanding of inflammatory mediated neuropathology. These metabolites may have originated either from upregulated TRYP catabolism within the CNS during inflammation, or may cross the blood-brain barrier after being systemically produced. It has been reported that CNS levels of KP metabolites increase independently of their systemic concentration during neuroinflammation, thus suggesting that KP metabolites can be present at upregulated levels locally within the CNS.

De novo NAD^+ Synthesis *via* the Kynurenine Pathway

QUIN is converted to nicotinic acid mononucleotide (NaMN) by the enzyme quinolinic acid phosphoribosyl

transferase (QPRT) in the presence of Mg^{2+}. Further transformation leading to the synthesis of the parent molecule of the pyridine nucleotide, NAD^+, appears to be nuclear, mitochondrial, and golgi specific, by nicotinamide mononucleotide adenyl transferase (NMNAT1, 2, and 3) in the presence of ATP to produce desamido-NAD^+. In the presence of glutamine desamido-NAD is amidated to the parent pyridine nucleotide, NAD^+, as the final product of the KP In addition to its *de novo* synthesis for TRYP, NAD^+ can also be synthesised from either one of three routes: (1) nicotinic acid (NA), which is then converted to NAD^+ *via* the three-step Preiss-Handler pathway; (2) the enzyme nicotinamide phosphoribosyl transferase (NAMPT) converts NM to nicotinamide mononucleotide (NMN) and then to NAD^+ by the action of NMNAT1, 2, and 3 in the presence of ATP, or (3) phosphorylation of nicotinamide riboside (NR) to NMN by NR kinases (NRKs) (Ratajczak et al., 2016) (**Figure 1A** and **B**). In spite of the potential for NAD^+ production from vitamins, the *de novo* synthesis of NAD^+ from TRYP appears to be more important than NAD^+ production from vitamins under normal physiological conditions.

Source of Tryptophan Catabolism During Neuroinflammation

The KP is not fully expressed in all brain cells. To date, the only cells in the CNS demonstrated to possess the enzyme 3-hydroxyanthranilic acid oxygenase (3-HAO) which generates QUIN are astroglial and microglia/macrophages/dendritic cells (Braidy et al., 2016). Therefore, the increase in TRYP catabolism observed during neuroinflammation must necessarily involve these two cell types.

IFN-γ is the primary activating factor of macrophage/microglial/dendritic cells in the CNS and elsewhere, increasing their antimicrobial activity through the modulation and upregulation of a variety of activities including, enhanced production of reactive oxygen species (ROS), increased nitric oxide synthase activity, upregulation of MHC antigens, secretion of cytokines such as interleukin (IL)-1β, IL-6, tumour necrosis factor-α (TNF-α), platelet activating factor (PAF), macrophage chemotactic protein (MCP-1), and secretion of other biologically active proteins such as complement pathway components. Indoleamine 2,3 dioxygenase (IDO) is the primary enzyme of the KP, and is potently induced by IFN-γ in both astrocytes and inflammatory cells leading to a marked increase in KP metabolites in these cells. IFN-γ activated microglial/macrophages/dendritic cells will readily catabolize TRYP through induction of IDO, producing significant amounts of metabolic neuroreactive intermediates such as KYN, anthranilic acid (AA),

3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA), and QUIN. The KP also leads to the production of the metal chelating agent, picolinic acid (PIC) (Schwarcz and Stone, 2016).

Astrocytes also readily degrade TRYP in response to IFN-γ treatment through the induction of IDO. KYN appears to be the main metabolite found in the supernatants of IFN-γ treated astroglial cells. However, significant but trace amounts of AA from an astrocytoma cell line, and QUIN, from human foetal brain cultures have also been reported (Guillemin et al., 2000).

It has been suggested that TRYP catabolism increased in some cells to decrease TRYP concentrations in the microenvironment thereby reducing the availability of this essential amino acid for microbial metabolism. However, this hypothesis has been challenged by others. One of the products of TRYP catabolism, 3-HAA, can function as an effective antioxidant, and potential nitric oxide synthase inhibitor. Production of this metabolite may therefore serve to reduce non-specific oxidative damage at the site of neuroinflammation induced by activated mononuclear phagocytes, and may explain the increased TRYP catabolism under these conditions (Krause et al., 2011). However, increased secretion of KYN and QUIN under these circumstances has not been confirmed. Moreover, IFN-γ induced IDO TRYP catabolism has been shown to increase cellular NAD^+ concentrations in an astroglioma cell line (Grant et al., 1999).

Despite the consolidated role of the classic KP metabolites (*e.g.*, KYN, KYNA, 3-HAA, and QUIN) during immune responses, it has recently emerged that cinnabarinic acid (CA) is crucial for immune system functions (Hiramatsu et al., 2008; Fazio et al., 2014). It has been shown that CA can be produced by stimulated human PBMCs. CA can also be produced *via* non-enzymatic reactions under oxidative conditions. In inflammatory cells such as neutrophils, co-expression of IDO and other enzymes involved in the formation of free radicals may divert the KP away from the production of QUIN, and instead towards CA over PIC or QUIN (Lowe et al., 2014). Therefore, concomitant implementation of NAD^+ levels could be enhanced by increased CA amounts.

Poly(ADP-ribose) polymerase and NAD^+ depletion: Double strand DNA breaks caused by excessive oxidative damage activate the PARP enzyme. Neuronal cells exposed to pathological concentrations of the excitotoxic neurotransmitter, glutamate show both an increase in intracellular oxidative stress and PARP activity. PARP is a protein modifying nucleotide polymerising enzyme found abundant in the nucleus, with approximately one molecule of enzyme per 1,000 base pairs. PARP, along with DNA dependent
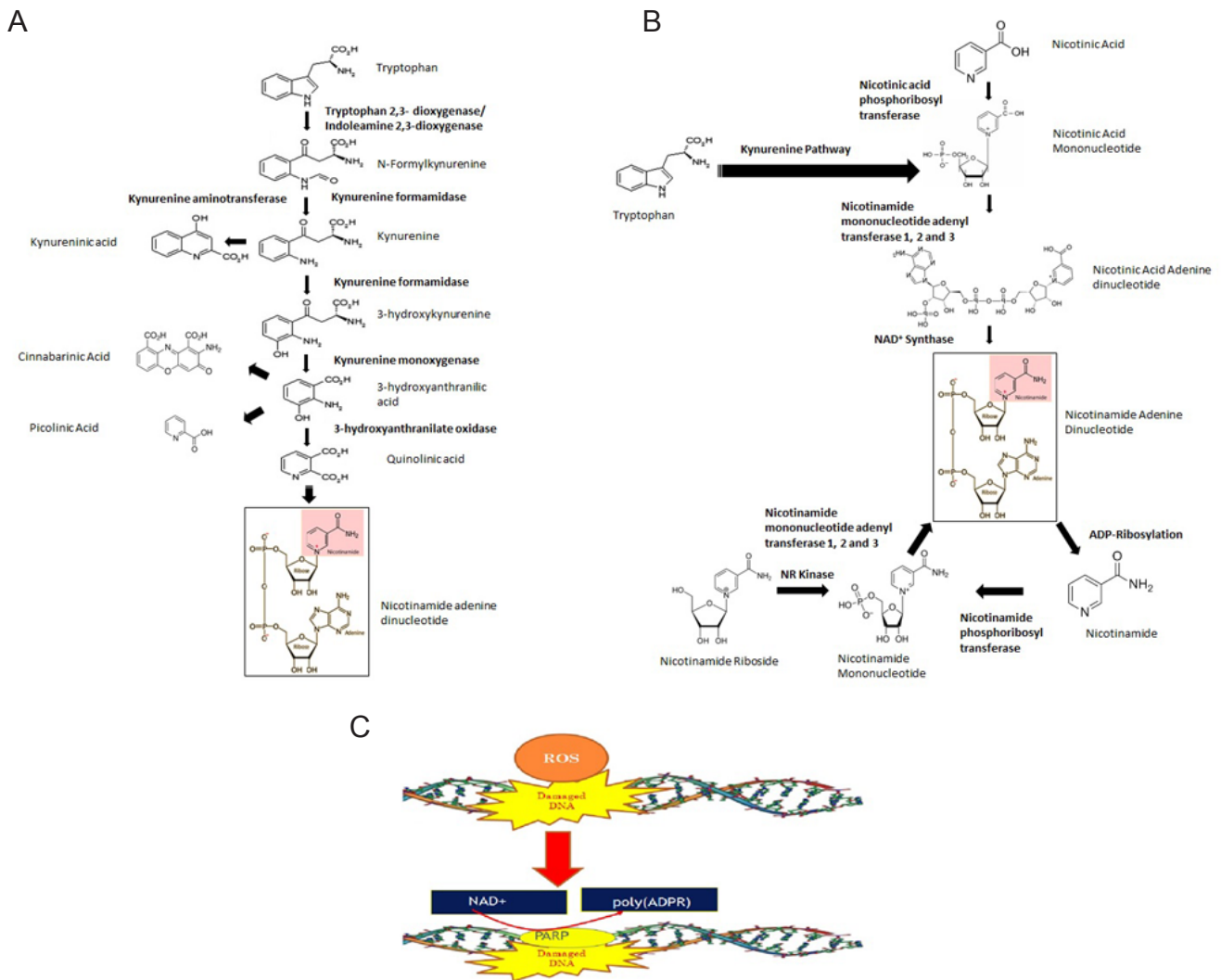
Figure 1 The nicotinamide adenine dinucleotide (NAD$^+$) metabolome.
(A) The *de novo* synthesis of NAD$^+$ from TRYP appears to be more important than NAD$^+$ production leading to the production of several neuroreactive compounds such as the neurotoxin and NMDA receptor agonist, quinolinic acid, and the NMDA receptor antagonist, kynurenic acid. (B) NAD$^+$ can also be produced by the salvage of nicotinic acid, nicotinamide and nicotinamide riboside. (C) PARP activity following DNA damage utilized NAD$^+$ as the essential substrate to repair damaged DNA. Hyperactivation of PARP due to accumulation of free radicals can lead to cell death by NAD$^+$ depletion, and energy restriction. NMDA: N-methyl D-aspartate; PARP: poly(ADP-ribose) polymerase; ROS: reactive oxygen species; TRYP: tryptophan.

protein kinases appears to play an important role in maintaining genomic integrity (Massudi et al., 2012). However, the precise physiological roles of PARP are not completely understood.

As a 'DNA nick sensor', PARP rapidly binds to DNA strand breaks and is activated. Activated PARP uses up NAD$^+$ (and NADP$^+$), exclusively as substrate to poly(ADP-ribosylate) itself and a number of nuclear proteins that are involved in the repair of DNA, releasing nicotinamide as a by-product. Recent evidence suggests that polyADP-ribosylation of histones or transcription factors may also be involved in nuclear receptor signalling (Morales et al., 2014) (**Figure 1C**).

A significant decrease in intracellular NAD$^+$ levels has

been reported in the brain and a variety of cell types as a result of DNA strand breaks and PARP activation following exposure to free radical generators, excitotoxins, infections, AD, and during inflammation or ageing (Abeti and Duchen, 2012; Braidy et al., 2014). Increased PARP activity resulting in decreased NAD$^+$ has been shown to decrease ATP and neurotransmitter levels in the brain, as well as cell lysis and death. Inhibition of PARP activity following oxidant injury has been shown to preserve NAD$^+$ and ATP levels, prevent cell lysis, although damage to the DNA was not prevented. Additionally, at low levels of oxidant, PARP$^{-/-}$ cells survived better than PARP$^{+/+}$ cells, suggesting that loss of NAD$^+$ may be a cause of cell death. Elevated levels of free radicals, pro oxidants, and excitotoxins have been reported

in inflammatory brain disorders, and in most cases, DNA damage has been observed. This suggests that NAD$^+$ depletion through PARP activation may play a crucial role in CNS dysfunction and pathology under these conditions (Massudi et al., 2012).

Conclusion

Immune activation of macrophage/microglial/dendritic cells results in a marked increase in their generation of ROS. Elevated extracellular fluid levels of these free radicals have been implicated in the cause of tissue damage during inflammation in several disorders. Damage to surrounding tissue, including astrocytes and neurons in the CNS, may occur in response to inflammation in the CNS. As well, the non-discriminating nature of these free radicals may induce damage to the activated macrophage itself. At least one study has reported an increase in PARP activity in IFN-γ activated macrophages, suggesting that DNA damage has occurred with a corresponding increase in the rate of NAD$^+$ catabolism.

The cellular immune response potentially increases NAD$^+$ catabolism in cells at the site of inflammation. A mechanism by which this important nucleotide could be regenerated appears to be essential to the continued viability of cells within this environment. Therefore therapeutic strategies targeted at inhibiting KP metabolism alone may not be sufficient for the resolution of symptomatic disease. However, inhibition of QUIN production may need to be coupled to the administration of a suitable NAD$^+$ precursor. This combination may then effectively reduce QUIN production, while maintaining optimal NAD$^+$ levels.

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