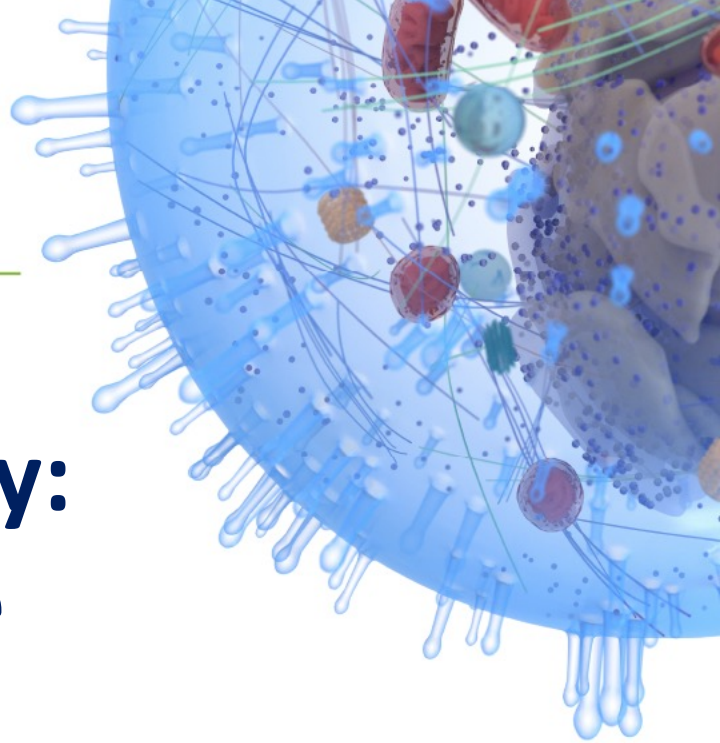

Tryptophan and kynurenine pathway: roles in inflammation and immune activation.

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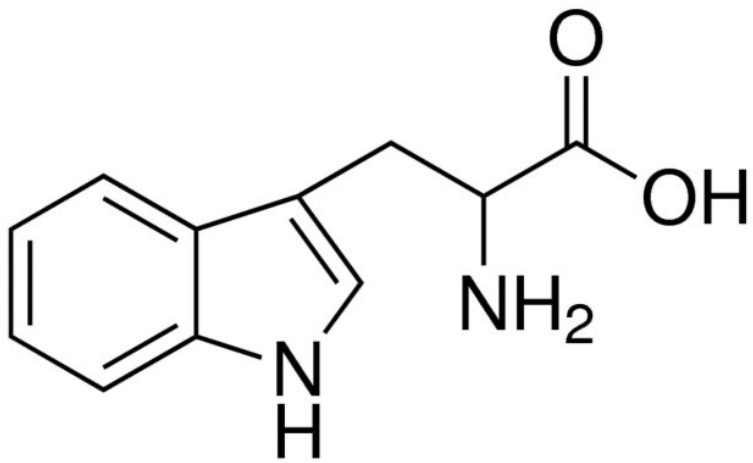
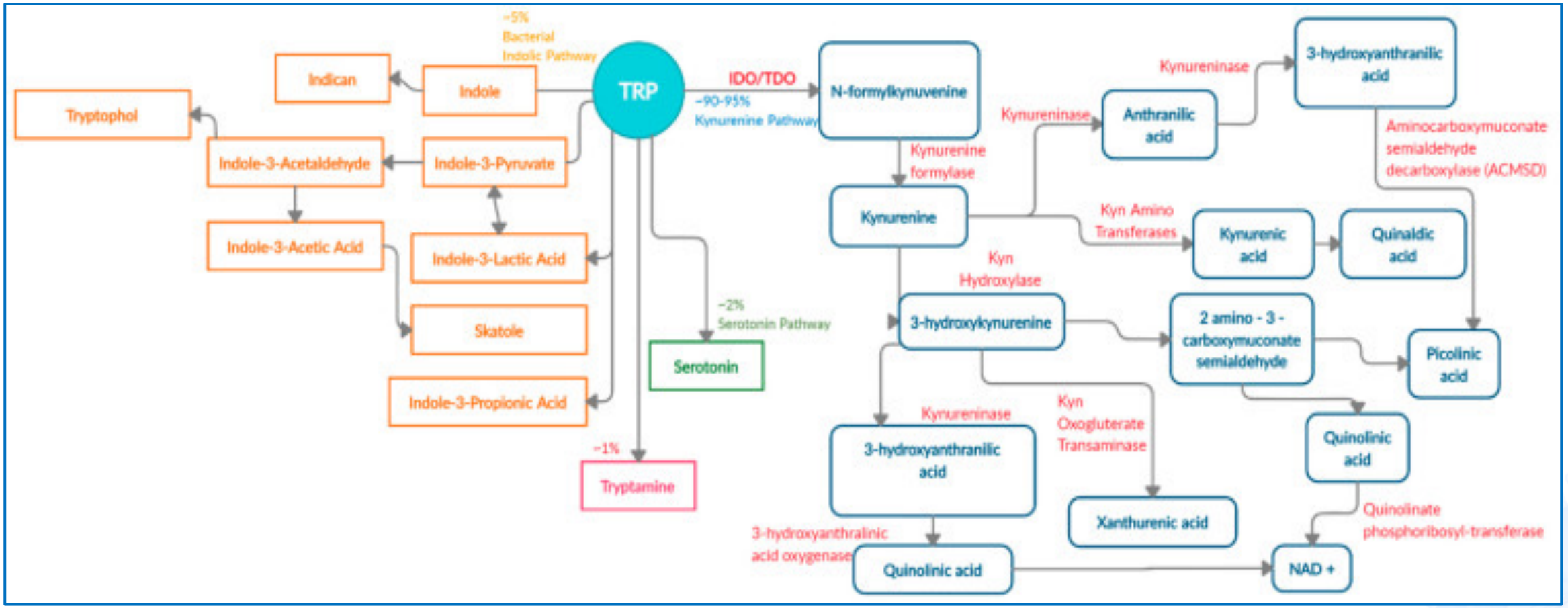
Tryptophan: overview.



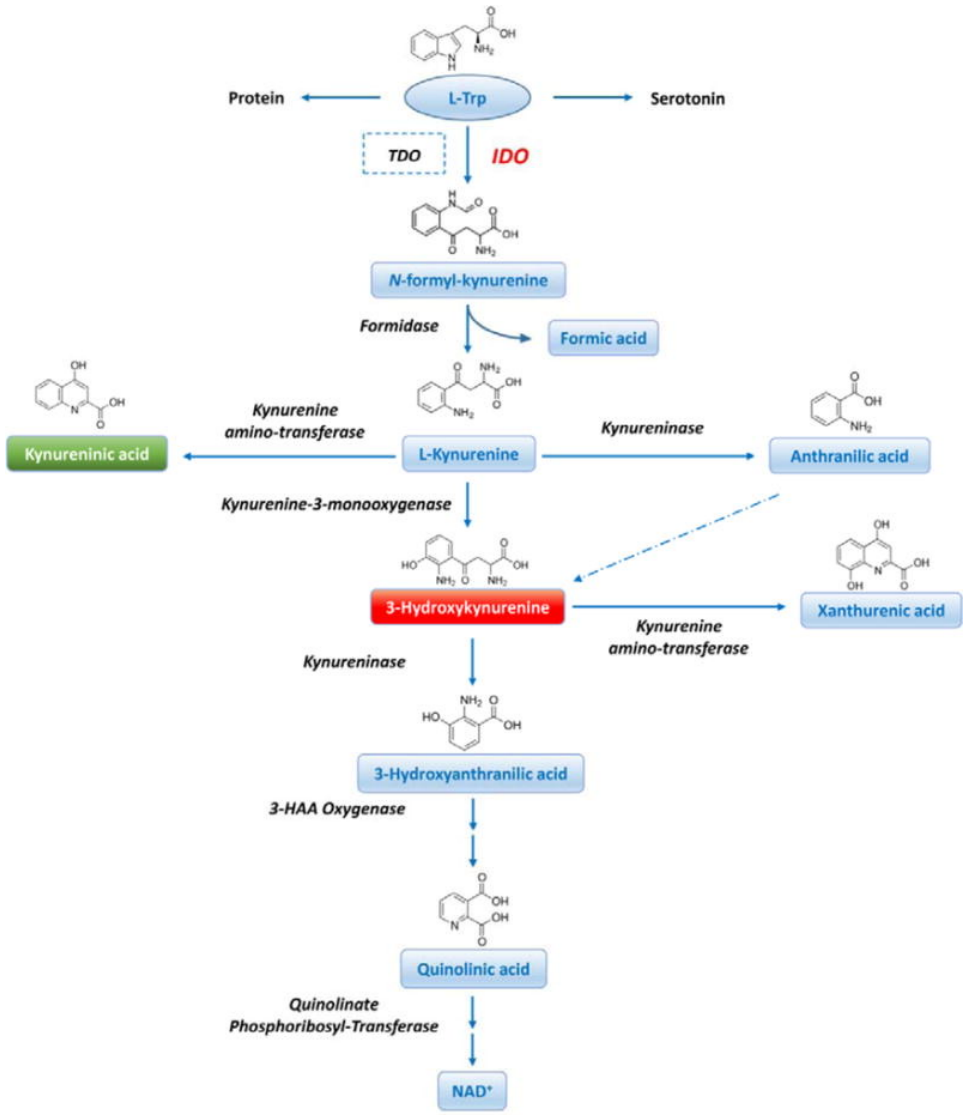
- **L-Trp** is an essential aromatic AA (one of the least abundant in our body) required for protein biosynthesis.
- The best dietary sources are meat like chicken and turkey, eggs, some fish (i.e. salmon), seeds, soya and dairy products.
- In NT, we often suggest to increase the intake of Trp-rich foods to improve mood and sleep as this AA is the precursor of **serotonin and melatonin.**
- In recent years, the focus has shifted towards the numerous bioactive Trp metabolites, as alterations in its metabolism have been associated with cancer, auto-immune diseases and neurological/ psychiatric disorders, among others.
- All these disease states appear to have an altered or amplified **Kynurenine pathway** activity.

Tryptophan: main metabolic pathways.



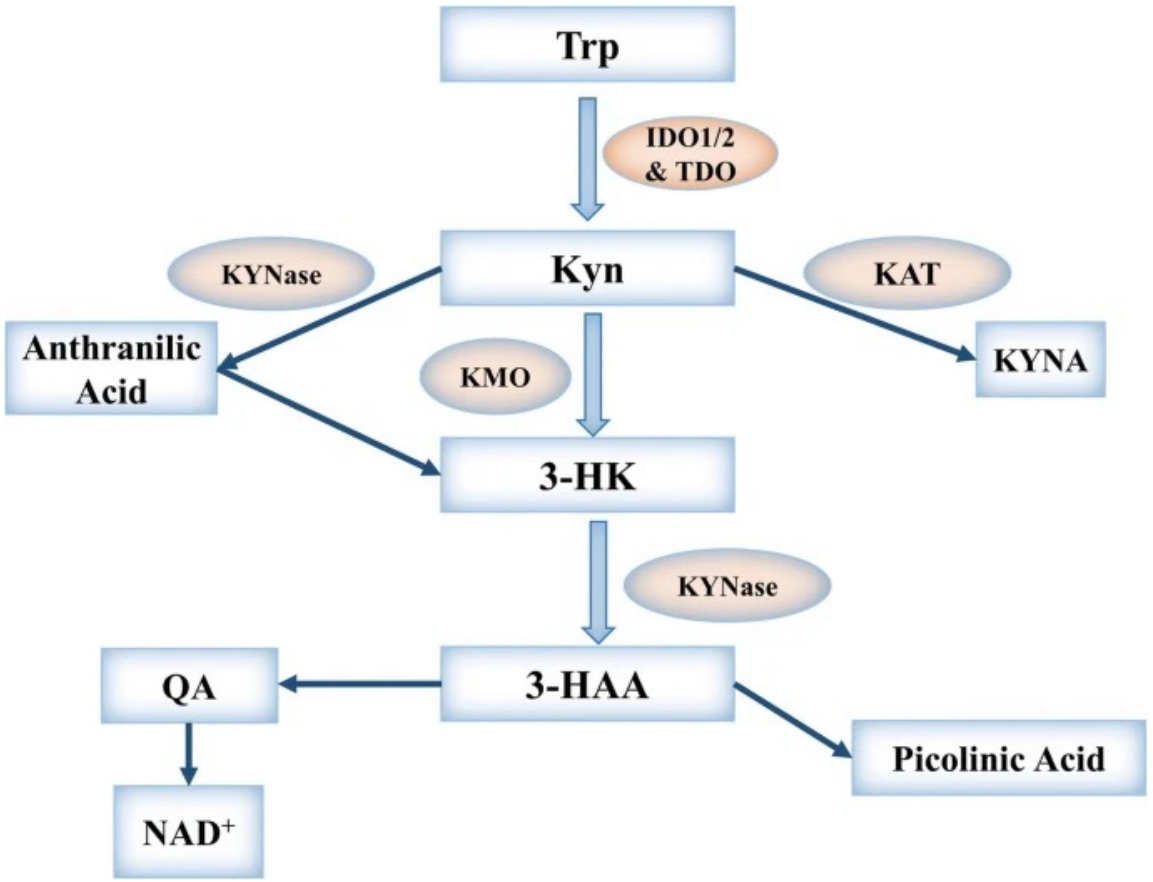
"Targeting Dietary and Microbial Tryptophan-Indole Metabolism as Therapeutic Approaches to Colon Cancer".

The Kynurenine pathway.



- 90 to 95 % of dietary Trp is shunted towards the kynurenine (KYN) pathway (in all mammals).
- The metabolites created in this pathway have profound effects especially on immune and nervous system
- Vast majority of KYN metabolism occurs in the liver (in physiological conditions)
- The end-product is **NAD+** (nicotinamide adenine dinucleotide), an essential co-factor for ATP production.
- Infections, inflammation (both chronic low-grade and acute) and immune activation up-regulate the KYN pathway
- Interestingly, in case of infections, the shunting of free Trp towards the KYN pathway decreases the availability of Trp for some bacteria. Therefore, this can be a direct way of limiting the virulence of some pathogens by manipulating the Trp pool
- However, some bacteria can themselves activate this pathway to evade the immune system. So, the Trp-KYN pathways is a double-edged sword in host-microbe interaction
- Overall, the activation of KYN pathway has **ANTI-INFLAMMATORY and IMMUNOSUPPRESSIVE effects.**
- Reduced Trp/ increased Kyn, alters the activation and balance of innate and adaptive immune cells towards a **tolerogenic milieu**

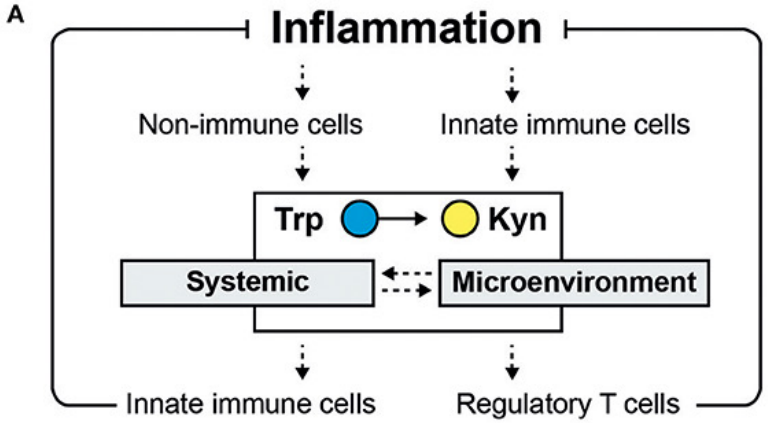
Key enzymes.

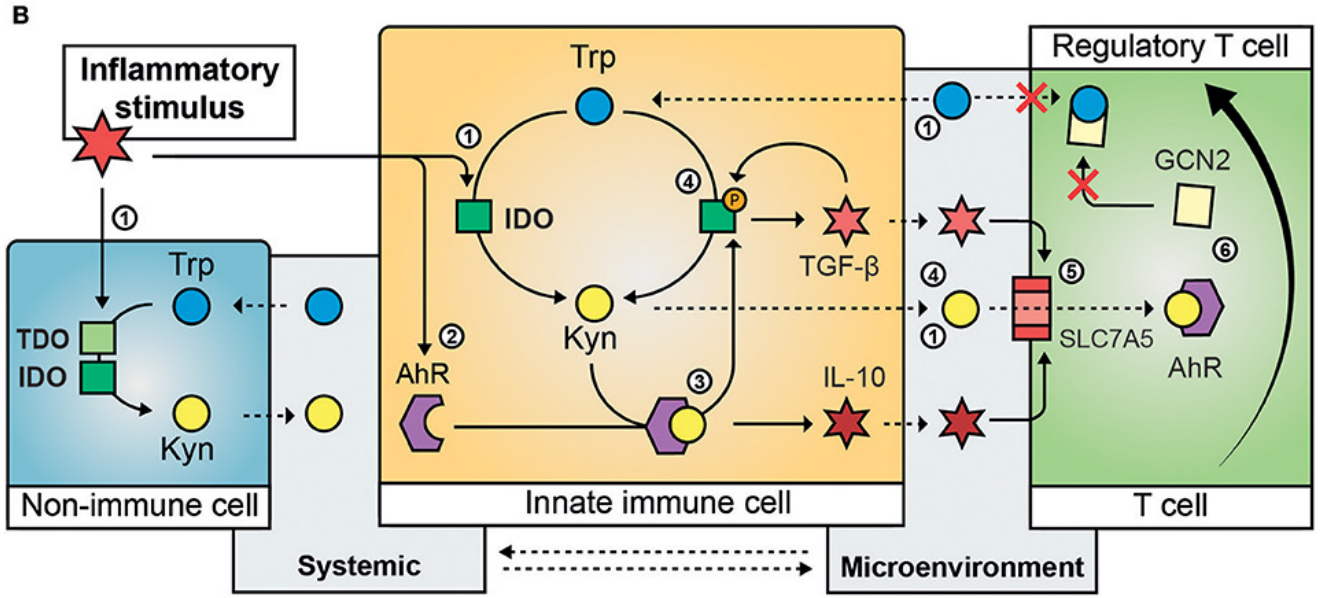


- The first and rate-limiting enzymes in Trp catabolism are **Tryptophan 2,3-dioxygenase (TDO)** expressed in the liver and **Indoleamine 2,3-dioxygenases** (IDO1 and IDO2) expressed in extra-hepatic tissues.
- In physiological conditions, Trp in mainly metabolized by TDO in the liver to produce Kyn, C -reactive proteins, haptoglobin, and fibrinogen.
- IDO plays a minor role in Trp metabolism under normal circumstances, but it is strongly activated **in response to interferons and other cytokines that are released upon inflammation.**
- **Interferon gamma (IFN-γ)** is considered the most potent IDO-activating cytokine
- Its effect is best-characterized in macrophages and dendritic cells (DCs) but is also evident in connective and epithelial tissue
- In general, pro-inflammatory cytokines but also LPS and PG2 can activate IDOs
- **KAT:** kynurenine aminotransferase, metabolizes L-KYN into **kynurenic acid**
- **KMO**: kynurenine 3-monooxygenase, metabolizes L-KYN into **3-hydroxy-kynurenine (3-HK)**
- **2 kynureninases:** one metabolizing L-KYN into **anthranilic acid** and one metabolizing 3-HK into **3-hydroxyanthranilic acid (3-HAA)**
- Final metabolites: **picolinic acid, quinolinic acid , nicotinic acid (vitamin B3) and finally NAD+**

Targeting the IDO1 pathway in cancer: from bench to bedside

KP and inflammation.

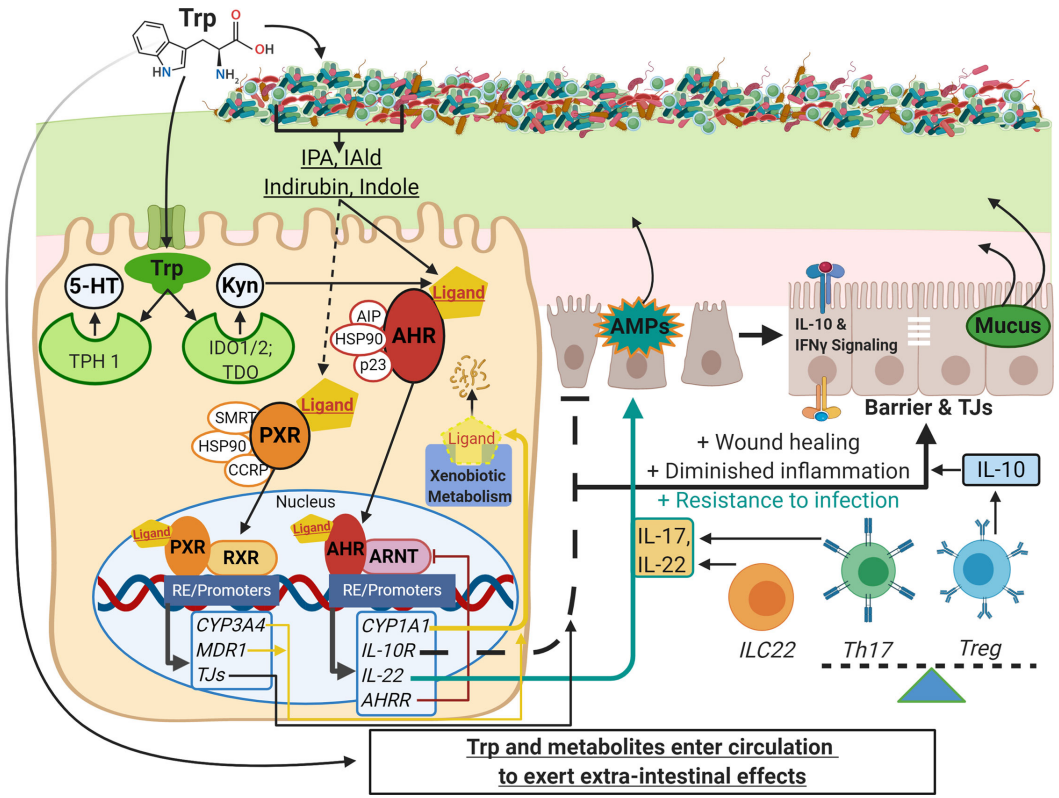




Tryptophan Metabolism in Inflammaging: From Biomarker to Therapeutic Target

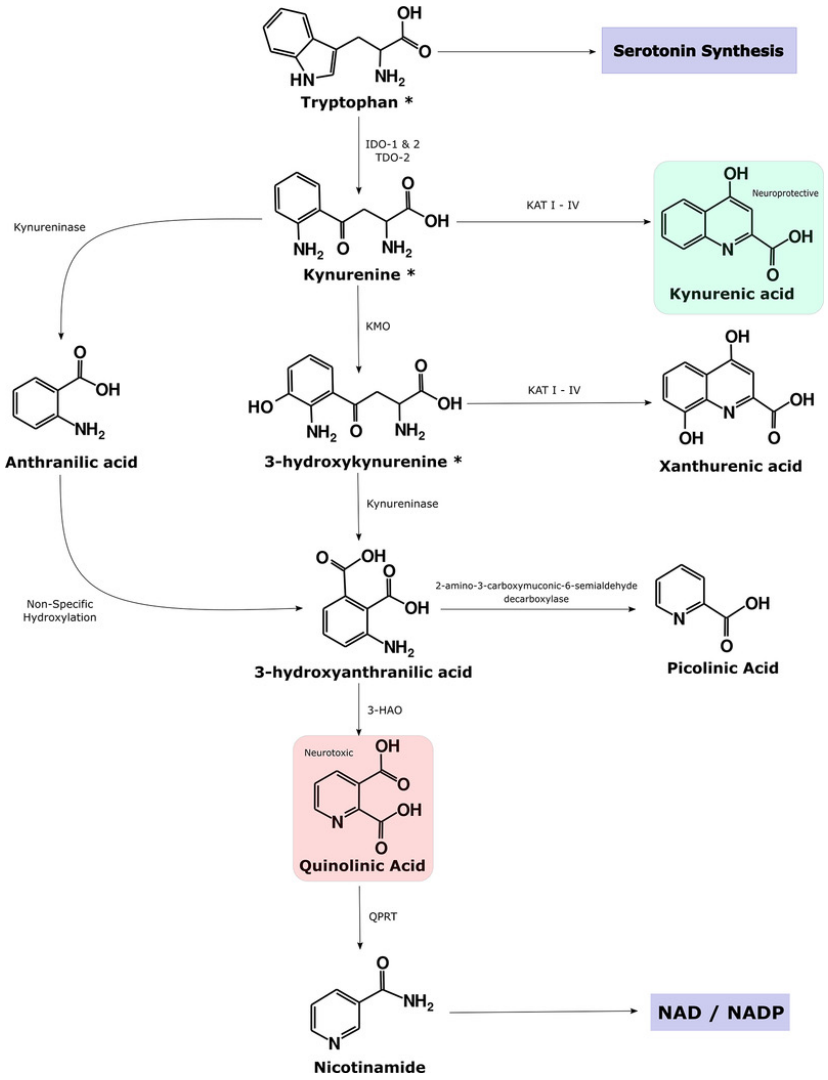
- IDO2 has a low catalytic activity so **IDO1** is the main enzyme activated in inflammation and immune activation.
- Under normal conditions, IDO1 expression regulates T cell proliferation to prevent tissue damage and reduce oxidative stress
- During inflammation, the expression of TDO is reduced while IDO1 expression is greatly increased to cause a reduction of activated T-cells, dendritic cells and natural killer cells and induce Th1 cell apoptosis to control excessive inflammation
- Trp depletion causes activation of KP, so IDO1-expressing cells like DCs and macrophages induce the kinase GCN2 (general control non-derepressable 2 stress kinase,), which in turn leads to the formation of anti-inflammatory cytokines (i.e. IL-10 and TGF-β)
- The final effect is the recruitment of Treg and prevention of T cell activation and proliferation

KP and AhR activation.



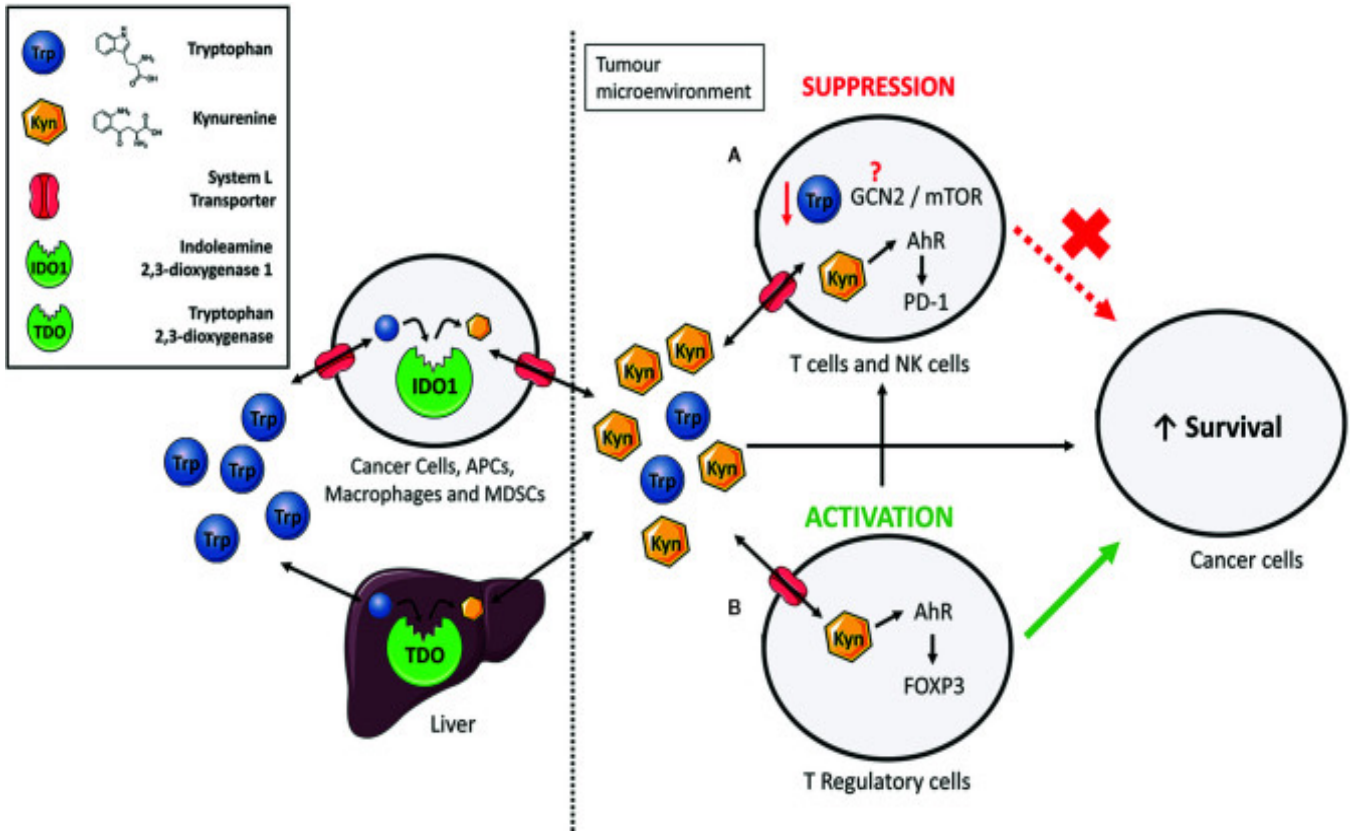
- All metabolites of the KP and the indoles, coming from Trp metabolized by gut bacteria, are ligands of the **Aryl hydrocarbon receptor (AhR)**
- AhR is a **transcription factor** belonging to a super family of TFs that control genes containing xenobiotic response elements (XREs), as well as non-XRE response elements (including estrogen receptor and retinoic acid receptors)
- Additional ligands of AhR are dioxins and dietary compounds, such as **DIM and I3C**
- Does the activation of AhR exert positive or negative effects? It depends on ligands and tissues! Findings are contradictory.
- For example, Ahr activation by dioxin induces organ-level dysfunctions caused by inflammatory factors such as **interleukin-6 , NF-kB and ROS production**
- **Polyphenols such as quercetin** and **resveratrol** are MODULATORS of AhR (they can both activate or inhibit AhR!)
- **Metformin** also suppresses AhR activation.
- Overall, the activation of AhR has **HORMETIC** effects: moderate activation has beneficial consequences, high activation can actually increases inflammation and oxidation. “Sola dosis facit venenum” !

KP key metabolites and their functions.



- **Kynurenic acid (KYNA):** generally considered to be **neuroprotective** as competitively inhibits ionotropic glutamate receptors at high concentrations but preferentially attenuates activity at the glycine co-agonist site of the NMDA receptor; it is also an agonist at an orphan G-protein-coupled receptor (GPR35), leading to a suppression of several inflammatory pathways. Agonist of AhR, **anti-inflammatory and anti-oxidant.**
- **Quinolinic acid:** opposite effects of KYNA, NMDA receptor agonist that can additionally inhibit reuptake of glutamate by astrocytes, leading to excitotoxicity, **powerful neurotoxin, it induces ROS production and inflammatory responses.**
- **Ratio between Trp and KYN** can be used as a biomarker of inflammation (increased KYN clearly indicates an inflammatory status)
- **Ratio between quinolinic acid and KYNA** may be useful to indicate neurotoxicity
- **AA** can have anti-inflammatory effects but high levels have been linked to neurodegeneration
- **XA** can have anti-oxidant and neuro-modulatory effects (but sometimes is pro-oxidant!)

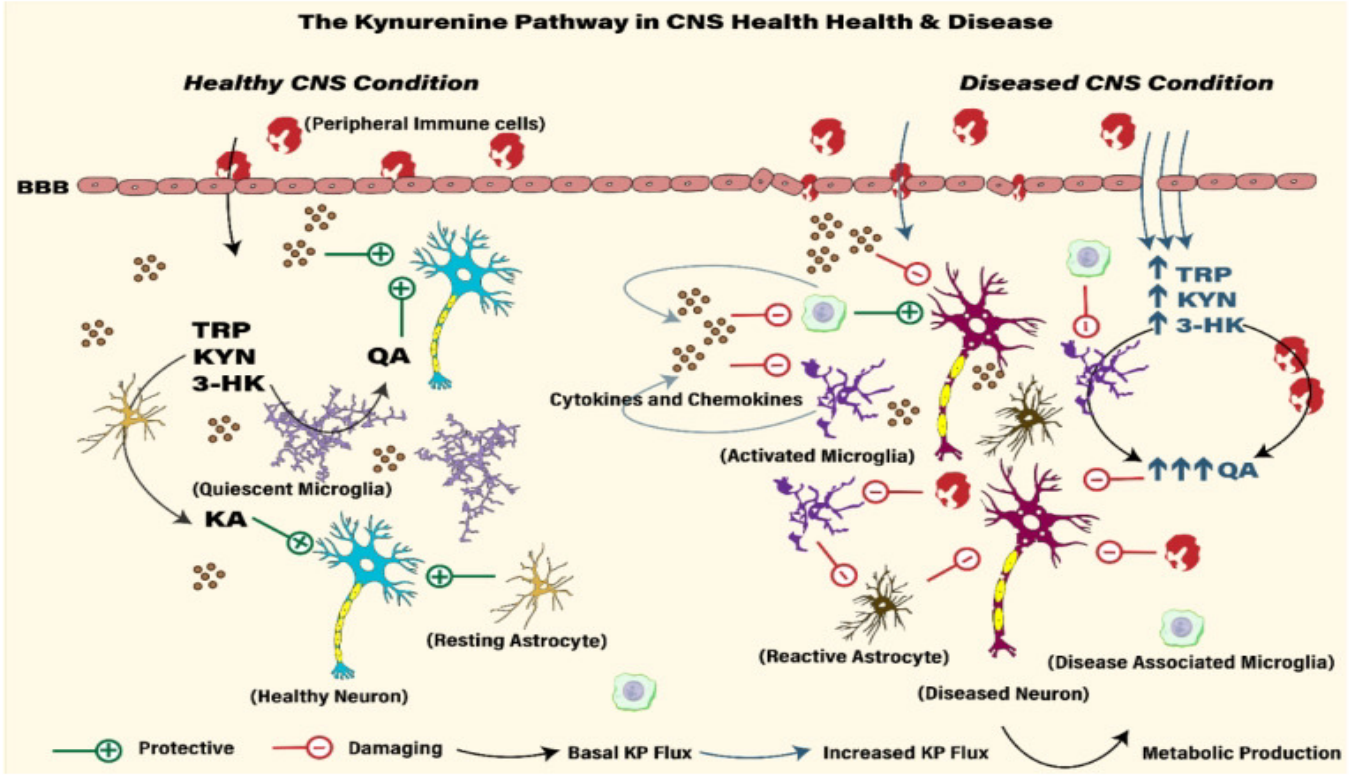
KP in cancer.



- Several cancers overexpress IDOs and TDO as this is a way to suppress immune activation, escape apoptosis and increase survival.
- Upregulation of IDO1 or elevated kynurenine levels associate with poor patient outcomes and resistance to immune checkpoint therapy such as anti-PD-1 inhibitors.
- IDO1 , KYN, TDO and AhR inhibitors are being used in cancer therapy with mixed results as Trp/KYN metabolites can be created by alternative pathways
- individual responses to anti-cancer therapies targeting the KP could be due to different **microbiota composition**
- Indoles can either promote immune activation or suppress it (in general, indoles have an anti-bacterial activity and improve IP and mucosal health).

Tryptophan: A Rheostat of Cancer Immune Escape Mediated by Immunosuppressive Enzymes IDO1 and TDO

KP in neurological/neuropsychiatric disorders.



Neuroinflammation and the Kynurenine Pathway in CNS Disease: Molecular Mechanisms and Therapeutic Implications

MDD: characterized by chronic inflammation (↑IL-6, TNF-α, CRP etc.), studies would suggest decreased levels of Trp and KYNA and increased KYN and QUIN (ketamine and omega 3 can help reduce both inflammation and KYN)

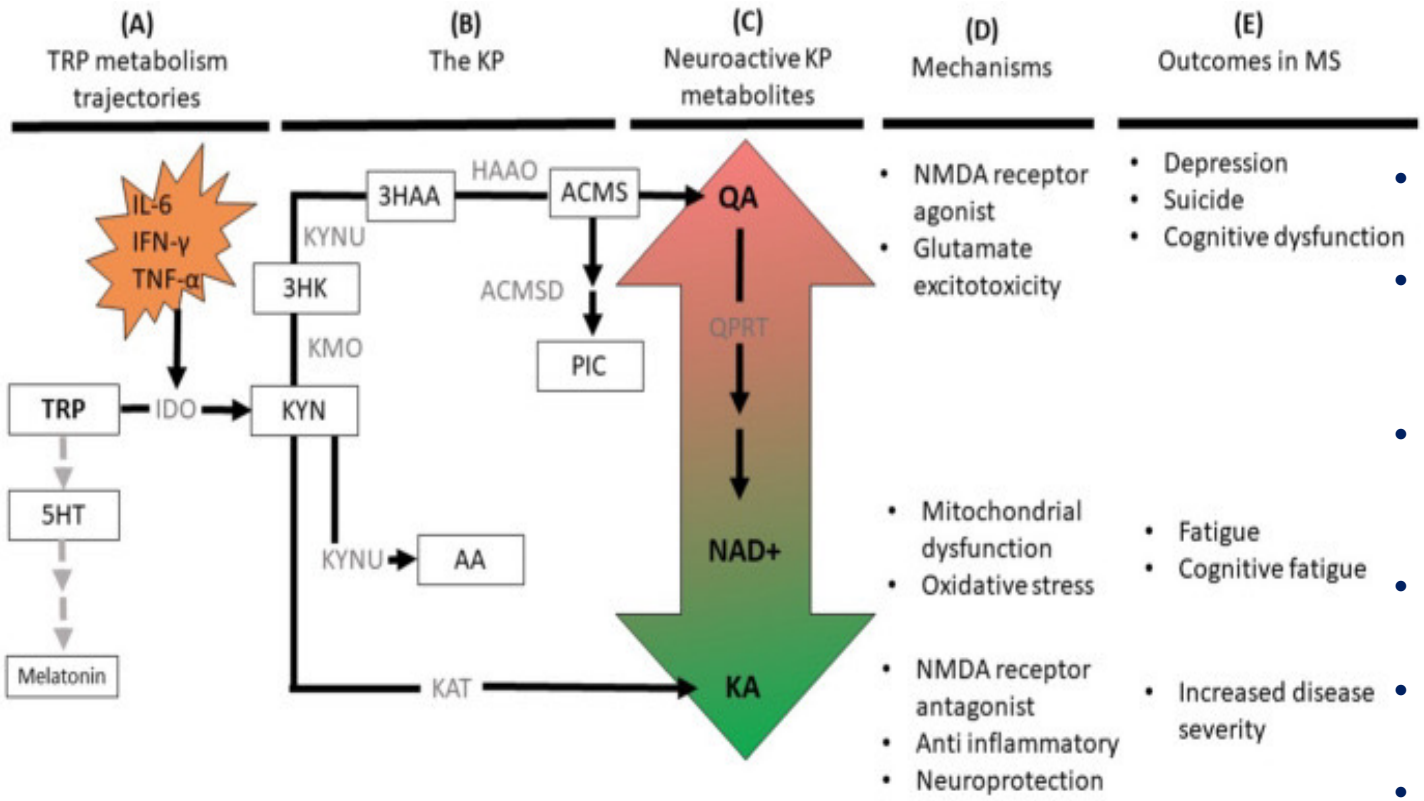
Schizophrenia: both elevated KYN and KYNA in CNS can alter glutamatergic and cholinergic neurotransmission with indirect increases in dopaminergic signaling (interestingly, in **BP** KYNA is decreased and 3-HK/KYN and 3-HK/KYNA ratio is increased)

ASD: significantly lower levels of KYNA, increased KYN/KYNA, high KYN and high QUIN

AD: decreased KYNA. KP metabolites could be used as biomarkers of AD. 5-HT (urine, serum), KYN (serum), KYNA (urine), L-TRP (urine, serum), and K/T ratio (urine) significantly lower in AD patients than in controls. But, KYNA is increased in CNS of AD patients!

PD: increased Trp, KYN and QUIN and decreased KYNA

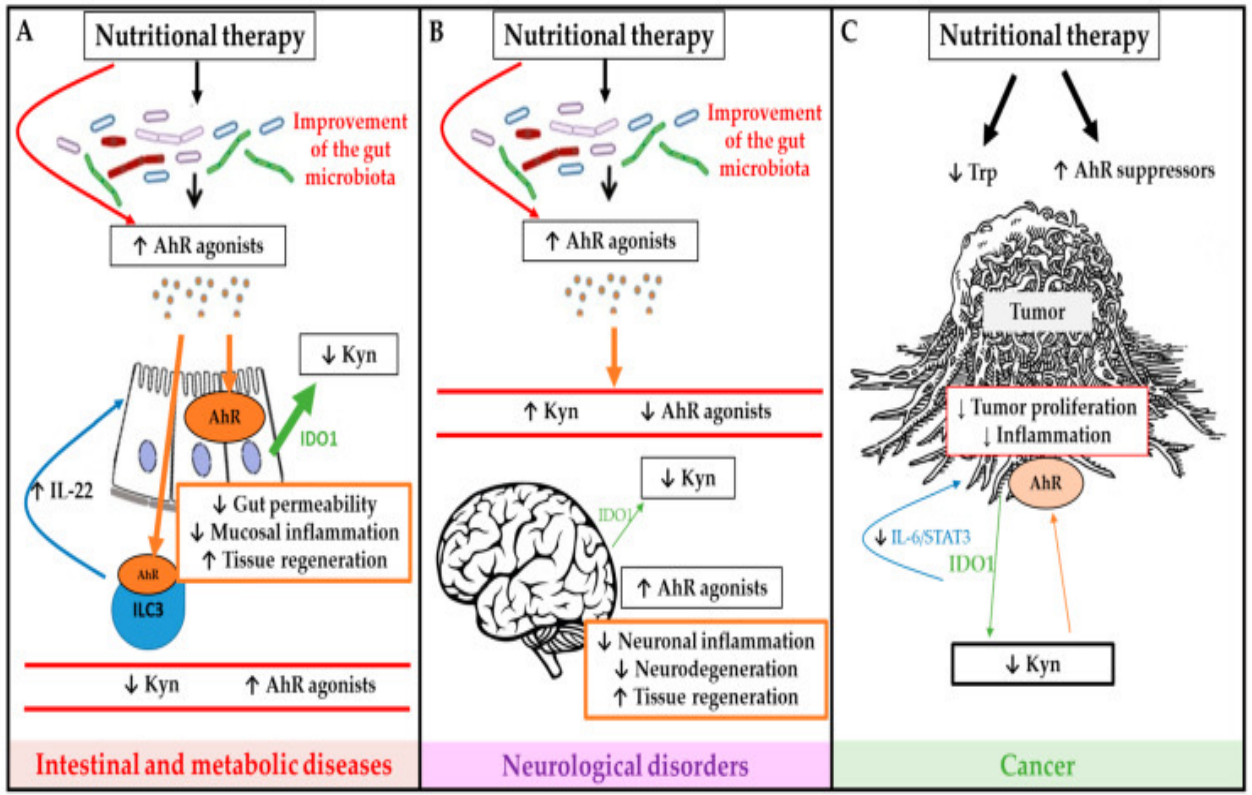
KP in auto-immune disease: the case of Multiple Sclerosis.



Exploring the roles of tryptophan metabolism in MS beyond neuroinflammation and neurodegeneration: A paradigm shift to neuropsychiatric symptoms.

- Pro-inflammatory cytokines are involved in the immune and inflammatory processes that drive MS etiopathogenesis and progression. Elevation in **IFN-γ, IL-6 and TNF-alpha** typically accompany clinical relapses.
- KP metabolite profile in MS is characterised by a **higher QA/KA ratio**, which is related to disease progression
- Besides the detrimental effects of QUIN on nervous system, KP activation can explain some of the neuropsychiatric symptoms associated with MS
- **Depression** is often a co-morbidity in MS and is strongly linked to inflammation. In fact, **NSAIDs** can improve mood in these patients.
- Activation of KP leads to decreased availability of 5-HT and therefore mood swings and sleep problems.
- IFN-β treatment, despite the positive effects on inflammation, can aggravate depression in MS patients.
- **Fatigue** is one of the most typical and debilitating symptoms in MS and can be a manifestation of cytokine-induced sickness behaviour. KP dysfunction can contribute to symptoms of fatigue in MS by disrupting mitochondrial function and increasing ROS. These negative effects are mainly due to increased QUIN.

Nutritional intervention to modulate the KP.



Nutritional Therapy to Modulate Tryptophan Metabolism and Aryl Hydrocarbon-Receptor Signaling Activation in Human Diseases

Diet, NT and supplements cannot directly influence the key enzymes and, therefore, metabolites in the KP. However, we can address the root causes and work on **inflammation and microbiome.** Moreover, as Trp becomes less available upon KP activation, we need to support the other metabolic pathways, so the serotonin/melatonin and the microbial indoles production.
Instead of supplementing 5-HT, which could in fact end up in the KP, I would suggest to supplement directly **melatonin,** in case of sleep disorders, poor **circadian rhythm** (i.e. in cancer, MS, auto-immune disorders etc.).
Supplementation with **SCFAs** may help support serotonin production in the gut
Indoles production can be improved with supplementation of **probiotics** (i.e. *Bifidobacterium* spp, *Peptostreptococcus russellii* and *Lactobacillus spp).*
Anti-inflammatory medicinal plants and/or supplements (i.e. **omega 3)** may be useful to control inflammation. These remedies should be personalized based on patients' characteristics.
Polyphenols can modulate AhR and the KP metabolites.

Conclusions.

- The KP metabolites profoundly impact the immune system, with an overall immunosuppressive effect.
- Although some of the KP metabolites and their ratio can be used as supportive biomarkers for immunological, inflammatory, neurological and neuropsychiatric diseases, we still need to investigate their roles as the effects may be contradictory.
- Enzymes inhibitors are promising agents for cancer and inflammatory conditions that present an overexpression of KP metabolites. However, controlling this metabolic pathway is extremely challenging.
- Diet and supplements can support KP modulation, by working on inflammation, Trp/serotonin/melatonin, microbiome modulation and AhR with dietary ligands.

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