

Evolutionary Views of Tuberculosis: Indoleamine 2,3-Dioxygenase Catalyzed Nicotinamide Synthesis Reflects Shifts in Macrophage Metabolism

Indoleamine 2,3-Dioxygenase Reflects Altered Macrophage Metabolism During Tuberculosis Pathogenesis

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Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme in conversion of tryptophan to kynurenines, feeding de novo nicotinamide synthesis. IDO orchestrates materno-foetal tolerance, increasing human reproductive fitness. IDO mediates immune suppression through depletion of tryptophan required by T lymphocytes and other mechanisms. IDO is expressed by alternatively activated macrophages, suspected to play a key role in tuberculosis (TB) pathogenesis. Unlike its human host, *Mycobacterium tuberculosis* can synthesize tryptophan, suggesting possible benefit to the host from infection with the microbe. Intriguingly, nicotinamide analogues are used to treat TB. In reviewing this field, it is postulated that flux through the nicotinamide synthesis pathway reflects switching between aerobic glycolysis and oxidative phosphorylation in *M. tuberculosis*-infected macrophages. The evolutionary cause of such shifts may be ancient mitochondrial behavior related to reproductive fitness. Evolutionary perspectives on the IDO pathway may elucidate why, after centuries of co-existence with the Tubercle bacillus, humans still remain susceptible to TB disease.

1. Introduction: An Overview of Tuberculosis

In this review, we note salient functions of indoleamine 2,3-dioxygenase (IDO) in immune tolerance, which may be relevant to tuberculosis (TB) pathogenesis. We highlight recent human studies supporting IDO as a host-derived biomarker of active TB. We describe recent developments in the growing field of immunometabolism and the relevance of oxidative glycolysis (Warburg-like metabolism) to tuberculosis. We take an evolutionary perspective to synthesize diverse streams of evidence, showing that IDO marks the confluence of reproductive immunology, mitochondrial bioenergetics, and infectious disease.

Active TB disease results from interactions between the host, microbe, and environment. Symptoms exist on a spectrum in which the organism may be latent or

quiescent, there may be sub-clinical mycobacterial replication without symptoms, or copious mycobacterial replication with florid TB disease, often resulting in death.^[1] During primary infection, patients may progress to disease or may remain asymptomatic. Following asymptomatic primary infection, reactivation disease can develop within a couple of years or even up to decades later. Once active TB has been cured, patients may develop reinfections or relapses. Human immunodeficiency virus (HIV) is a potent risk factor for symptomatic TB and the HIV epidemic has greatly aggravated the TB burden.^[2]


Due to limitations of microbial assays for the *Mycobacterium tuberculosis* (*M. tuberculosis*) organism, the search for host-derived immunological TB biomarkers has been ongoing for decades. Assays that measure activity of the enzyme, indoleamine 2,3-dioxygenase-1 (hereafter referred to as IDO), have recently been proposed as additional TB diagnostic tools.^[3]

Blood-based immunological assays such as interferon-gamma release assays (IGRAs) have performed well to diagnose prior exposure to *M. tuberculosis*. IGRAs were based on the principle of distinguishing a memory immune response to Tuberculosis

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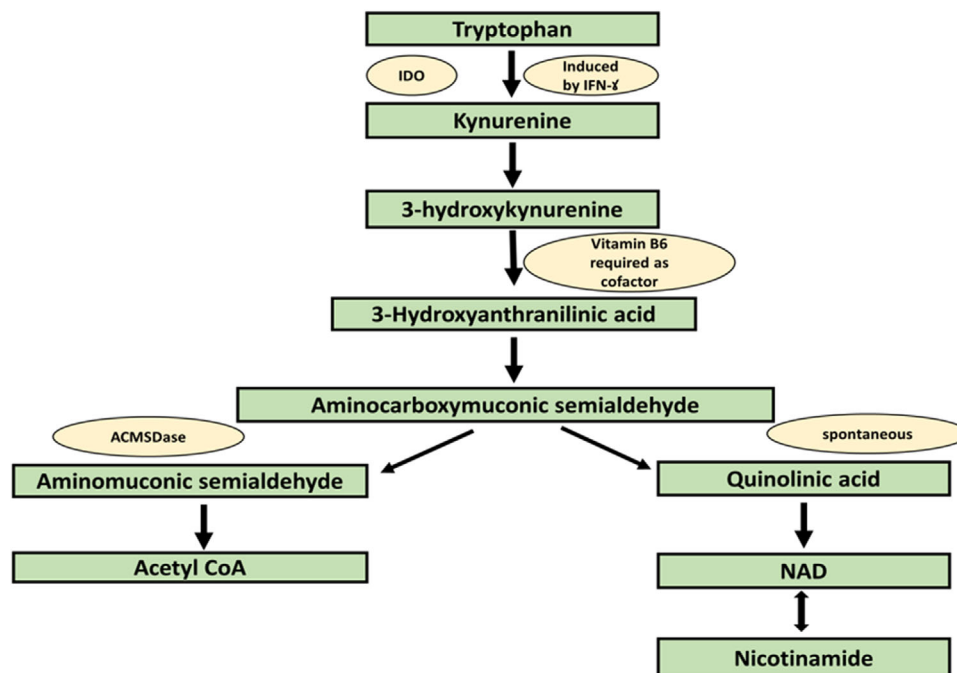


Figure 1. The tryptophan–kynurenine–nicotinamide pathway. IDO, indoleamine 2,3-dioxygenase; NAD, nicotinamide adenine dinucleotide; ACMSDase, α -aminomuconate- ϵ -semialdehyde decarboxylase. Image based on concepts found in refs. [7, 74].

antigens from a primary response.^[4] In contrast to the laborious study of interferon-gamma for TB diagnostics, the immunomodulatory enzyme IDO is only recently starting to attract the spotlight in the Tuberculosis field.

In recent years, IDO has been a rising star in diverse fields such as cancer immunology, transplantation, neurobiology and infertility medicine. For tuberculosis, however, IDO has been a little-known actor waiting in the wings.

2. IDO Catalyzes the Kynurenine-Tryptophan-Nicotinamide Pathway

Tryptophan is an essential amino acid and cannot be synthesized by humans. Tryptophan is used either for synthesis of neuroactive metabolites such as serotonin and melatonin, or catabolized to kynurenines.^[5] IDO is a cytosolic enzyme with a haem prosthetic group.^[6] IDO is the rate-limiting enzyme in the conversion of tryptophan down the kynurenine pathway towards de novo synthesis of nicotinamide, **Figure 1** (reviewed in ref. [7]). Nicotinamide is essential for cellular metabolism, as part of nicotinamide adenine dinucleotide (NAD), which shuttles between oxidized (NAD⁺), and reduced (NADH) states.

Kynurenines are metabolites with toxic effects on microorganisms as well as on T lymphocytes. Generation of kynurenines via IDO activation has a direct antimicrobial effect as well as a host immune-suppressive effect. By consuming tryptophan and decreasing serotonin synthesis, IDO activity modulates mood and promotes depression.^[5,8–9]

An important site of production of IDO is the placenta, where IDO mediates tolerance to the foetal allograft. At the placenta,

IDO inhibition results in miscarriage of allogeneic pregnancies (which are genetically mismatched at the major histocompatibility locus) but not syngeneic fetuses (matched at the major histocompatibility locus).^[10]

IDO is transcribed in various immune tissues including the tonsils, lymph nodes, and the spleen, resulting in multiple immune suppressive effects.^[6] IDO is thought to be mostly confined to mammals.^[11] A variant of IDO, known as IDO2, is found in lower vertebrates, mammals and humans and contributes in a minor way to extrahepatic tryptophan conversion to kynurenines.^[11] A related enzyme, tryptophan 2,3 dioxygenase (TDO) is found in the liver and mediates intrahepatic conversion of tryptophan to kynurenines.^[11] Through tryptophan conversion to kynurenines, IDO activity depresses T cell activation by starving T cells of tryptophan,^[12–13] and IDO activity can result in the generation of regulatory T lymphocytes.^[14–15]

Strangely, IDO gene transcription is induced by interferon-gamma.^[5] As interferon-gamma is typically described as a pro-inflammatory cytokine, this counterintuitive association suggests that IDO induction is part of a negative feedback loop dampening down immune response.^[5] As IDO is stimulated by pro-inflammatory cytokines, IDO activity contributes to sickness-induced behavior during fever, via tryptophan depletion.^[8,16–17]

Kynurenine can act directly as a ligand for the aryl hydrocarbon receptor, which is a transcription factor controlling induction of immune response genes and cytochrome P450 genes.^[5] The aryl hydrocarbon receptor integrates sensing of toxins, microbial factors, and host kynurenine metabolites for induction of immune genes.^[18] Kynurenines generated via IDO activity bind to the aryl hydrocarbon receptor, which in turn mediates

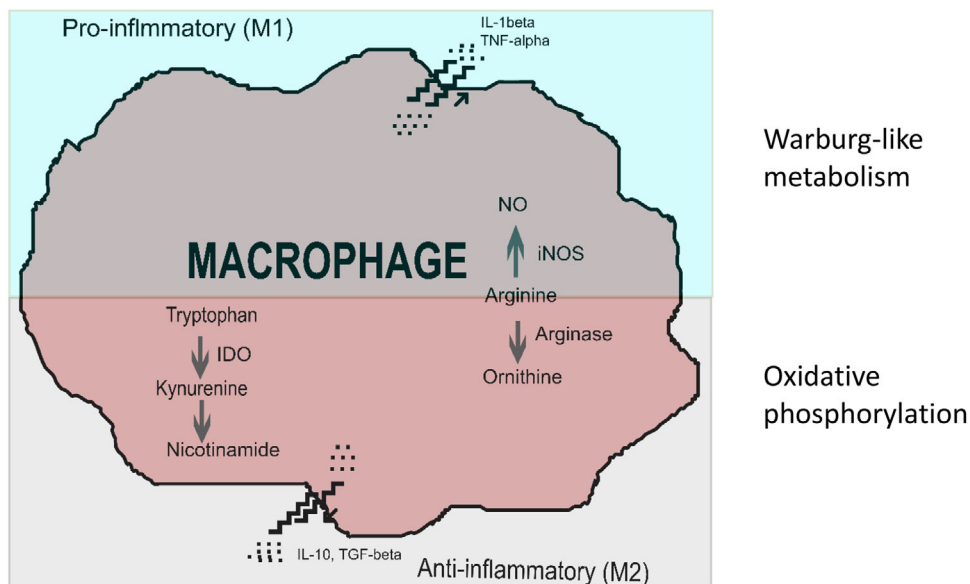


Figure 2. IDO is a key difference in the metabolism of immunosuppressive (alternatively activated or M2) macrophages compared with pro-inflammatory (classically activated or M1) macrophages. Arginine metabolism also differs between the two phenotypes of macrophages. Image based on concepts found in refs. [67, 116]. The two macrophage phenotypes differ in their metabolism, M1 macrophages show Warburg-like metabolism (aerobic glycolysis) and M2 macrophages use oxidative phosphorylation.^[39,87–90]

generation of FOXP3-expressing regulatory T cells, which have an inhibitory role on other cells of the immune system.^[14–15,19] Interestingly, pigment from *M. tuberculosis* can also bind directly to the aryl hydrocarbon receptor,^[20] presumably inducing the same downstream effects as kynurenines.

In summary, activation of the IDO enzyme during inflammation causes an increase in pathway products, such as kynurenines and nicotinamide, while decreasing concentrations of the pathway's substrate, tryptophan.

3. Alternatively-Activated Macrophages Produce IDO

Immune cells, particularly macrophages, are sites of IDO production. For *M. tuberculosis*, the macrophage is a key niche, able to harbor dormant bacilli, becoming the site of bacilli reactivation. IDO expression in macrophages causes suppression of T cells through cell cycle arrest.^[13,21] IDO induction is able to switch macrophage phenotype from what can simplistically be thought of as pro-inflammatory (or M1 macrophages), to an anti-inflammatory or tolerogenic phenotype, often termed alternatively-activated or M2 macrophages.^[22–24] IDO is also expressed by dendritic cells, where it is thought to confer them with a regulatory (tolerogenic) phenotype^[14–15,25] The tolerogenic or alternative pathway of macrophage or dendritic cell activation is depicted in **Figure 2**.

M2 macrophages are described as those involved with tissue repair, fibrosis, and healing.^[13,23] As fibrosis curtails spread of mycobacteria by circumscribing the TB granuloma, M2 macrophages may be pivotal in disease outcome during TB. Macrophage phenotype may mediate immune tolerance to

M. tuberculosis, in which the human host and microbe exist in a state of equilibrium or symbiosis.

4. Evolutionary Perspectives on Immune Tolerance to Tuberculosis

Evolutionary medicine, as defined by “grandfathers”, Nesse and Dawkins, seeks to ask and answer questions related to ultimate causes of pathology (“why” questions), as opposed to proximate causes of disease (“how” questions).^[26–27] Much medical research focuses on proximate mechanisms of disease: which cytokines, proteins, and mediators are associated or causative of particular symptoms in response to particular pathogens. Why such cytokines, proteins and mediators should act in such a coordinated manner to induce disease, despite millennia of co-evolution with the organisms, may, however, be the more pertinent questions.

Contrary to common perception, Darwinian survival of the fittest does not imply natural selection of those who are stronger, healthier, or more disease resistant than their neighbors. If the fittest, strongest, healthiest individuals alive were unable to reproduce, their genes for vigor would not be passed on to any future generation. In order to answer “why” humans remain susceptible to a particular disease despite millennia of natural selection, Nesse and Dawkins emphasize that “selection maximizes reproduction, not health”. The corollary is that “a trait that increases reproduction will tend to spread, even if it harms health”.^[26]

An intensified focus on mediators and mechanisms of reproduction should thus benefit infectious disease researchers. If we better understood the immune role-players needed for

reproduction, we may better understand why such mediators are essential throughout life, despite perhaps making us vulnerable to disease. IDO is intriguing from this perspective, as IDO is pivotal both for reproduction and as a key tolerogenic switch in the immune response.

Reproductive biology and infectious disease biology intersect at the concept of “immunological tolerance”. The term tolerance can be defined in various ways. Plant biologists distinguish host resistance and host tolerance. To plant biologists, host resistance implies the ability to limit parasite burden, while host tolerance implies the ability to limit the harm caused by a particular parasite burden.^[28–29] To clinical immunologists, tolerance is usually defined as a state of immune unresponsiveness specific to a particular antigen, and which has been induced by prior exposure to that antigen.^[29–30]

While immunological tolerance is experimentally and therapeutically demonstrable in many models, including allergen desensitisation, and transplantation tolerance, laboratory methods to study and characterize immune tolerance remain inadequate despite decades of research.^[31–32] Even our immunological “language” does not well characterize homeostasis between host endogenous and exogenous microbial flora.^[33–34] What distinguishes a commensal organism from a pathogen remains a murky question: for example, many organisms, such as *Neisseria meningitidis* or *Streptococcus pneumoniae*, are able to switch between commensal and pathogenic behaviors.^[35–37] An improved understanding of immune tolerance may lead to insights into infectious disease pathophysiology, particularly for *M. tuberculosis*.^[38] *M. tuberculosis*'s natural history of latency and reactivation is closely linked with waxing and waning of host immunity.

IDO is a strong candidate for a master-regulator of immune tolerance. There are two relevant evolutionary perspectives that may be illuminating. The first is the link between the IDO pathway and host reproductive fitness, via the mechanism of action of IDO on fertility. Any pathway by which IDO is upregulated may have secondary benefits on reproductive fitness of the human host, with a resultant benefit on propagation of the human species. Thus, genes predisposing for susceptibility to diseases in which IDO is upregulated, may be amplified in the human population, despite deleterious pathology or increased death rates caused by the disease itself.

The second perspective to consider is the very nature of eukaryotic life itself, questioning why metabolism may differ during infection with pathogenic versus non-pathogenic organisms. The field of immunometabolism has made some extraordinary advances in recent years, converging on the role of mitochondria in the balance between health and disease.^[29,39–41]

5. Dietary Deficiency of Tryptophan and Nicotinamide Predispose Humans to Tuberculosis

TB has plagued humans since prehistoric times. Skeletal abnormalities in Egyptian mummies document Potts disease (TB of the spine), while TB DNA has been found in Iron Age skeletons dating back to 400 BC.^[45] The disease, termed the “white plague” ravaged Europe in the 1800's, resulting in well publicized literary works featuring “consumption”.^[45] Why human populations

have not become more resistant to TB over time remains a conundrum. Children and young adults are well represented within those dying from TB; therefore, centuries of co-evolution should have decreased reproductive fitness of the susceptible human population, resulting in reduced transmission of TB-susceptibility host genes. Persistence of the organism in one third of the global population can therefore be hypothesized to harbor strong selective benefits, either in host survival until reproductive age or in terms of reproductive fitness itself.

The IDO pathway of tryptophan conversion to kynurenines is integrally linked with generation of nicotinamide. A precursor of nicotinamide is niacin, otherwise known as Vitamin B3.^[46–47] Nicotinamide must either be consumed through the diet or synthesized de novo from tryptophan. Poor diets, particularly poor consumption of animal products, lead to deficiency states such as pellagra. *M. tuberculosis* is an organism that can synthesize tryptophan^[49] and nicotinamide adenine dinucleotide.^[50] Hill and colleagues have postulated that dietary changes over the last centuries have created an evolutionary benefit to *M. tuberculosis*-infected individuals, where *M. tuberculosis* infection (presumably latent infection) compensates for dietary deficiency of tryptophan or nicotinamide.^[51]

While it is well known that TB disease rates are associated with poor socioeconomic status, Hill and colleagues showed inverse correlation during the pre-antibiotic era in 19th century England, specifically between meat intake and TB deaths.^[51] Although correlations are not causations, such hypotheses may guide future research questions. Hill and colleagues stressed the importance of tryptophan and nicotinamide for human health, development, and fertility in general. Hill and colleagues did not remark, however, on centrality of IDO to reproductive immunology and fetomaternal tolerance. The integral role of IDO within reproduction adds weight to possible evolutionary benefits that could accrue from commensal infection with an organism that can enhance IDO expression.

6. IDO Activity is Elevated During Human Tuberculosis

Multiple animal and human studies have shown convincingly that IDO activity is increased during symptomatic TB. Regarding human studies, Almeida and colleagues found elevated IDO expression in induced sputa of patients with TB disease compared with patients with other lung diseases and controls.^[52] The elevated IDO levels decreased more than 500-fold within two weeks of TB treatment. In patients with TB pleurisy, Li and colleagues deduced that high IDO levels in pleural fluid led to suppression of T cell activity.^[53]

In elderly Japanese patients, Suzuki et al. found IDO activity to be an independent prognostic factor for death versus recovery from TB.^[54] In subsequent work, Suzuki et al. showed that the kynurenine/tryptophan ratio in serum or pleural fluid was significantly higher in TB patients than in patients with other lung diseases.^[55] Epithelioid granulomata in pleural tissue showed high IDO expression by immunohistochemistry, suggesting involvement of IDO in pathogenesis of TB disease.

In a TB endemic area with high HIV prevalence, we showed that plasma IDO activity was significantly higher in patients with

active Tuberculosis and HIV co-infection than in HIV-infected controls, including those with pneumonia.^[56] Plasma IDO was elevated up to six months prior to TB diagnosis, suggesting that elevated IDO activity could predict disease onset.

Metabolomics studies have identified altered pathways of amino acids in active TB disease.^[57–59] Feng and colleagues report upregulated kynurenine and quinolinic acid in the top 12 altered metabolites amongst 400 small molecules explored in the serum of TB patients compared with patients with other lung diseases.^[57] Weiner et al. identified significantly elevated kynurenine in the serum of patients with active TB and showed that cultured macrophages upregulated IDO gene expression following infection with *M. tuberculosis*.^[59]

In a prospective study in Sub-Saharan Africa, kynurenine increased a few months prior to time of active TB.^[60] In a different setting, of 351 cerebrospinal fluid metabolites studied by Van Laarhoven et al., tryptophan was amongst the top five differing between patients and controls. TB patients had lower cerebrospinal fluid tryptophan, but very low levels also correlated with survival.^[61]

In addition to human studies, supportive work using animal and in vitro models has highlighted the relevance of IDO activity in TB pathogenesis^[17,49,62–64] but the models will not be reviewed in detail here. Notably, not all human metabolomic studies have identified kynurenine or tryptophan as having altered levels in TB. Lack of detection or mention in some metabolomic approaches may perhaps be due to analysis of individual metabolites rather than the ratio of kynurenine to tryptophan. Use of product-to-substrate ratio may magnify effects not statistically apparent for either analyte alone.^[58,65–66]

In HIV infection, even without concurrent Tuberculosis, a large body of work (reviewed by Adu-Gyamfi et al.^[67]) has illustrated elevated levels of IDO activity. Diversion of tryptophan away from serotonin synthesis towards formation of kynurenine by-products (which are largely neuro-excitatory) correlates with HIV-associated dementia and cognitive decline as well as disease progression to AIDS.^[68–71] The synergistic interaction between HIV and Tuberculosis speculatively appears to converge on IDO activity mediating decreased tryptophan and increased kynurenines.

7. Human and Microbial Nicotinamide Synthesis Pathways Interact

Nicotinamide is essential to both host and mycobacterium. Interestingly, *M. tuberculosis* can synthesize tryptophan under stress conditions.^[49] IDO-mediated tryptophan depletion is therefore insufficient for mycobacterial control through substrate depletion.

Furthermore, *M. tuberculosis* can synthesize NAD⁺ de novo, starting from aspartate rather than tryptophan as a precursor molecule, in a similar fashion to other prokaryotes.^[72] *M. tuberculosis* can also utilize a salvage pathway to regenerate NAD⁺ from nicotinamide.^[50,72] In fact, production of niacin was the principle of a phenotypic test used prior to modern molecular methods to identify *M. tuberculosis* from culture.^[73]

Intriguingly, nicotinamide itself suppresses TB symptoms. Nicotinamide was discovered serendipitously in the 1940s to cure

TB.^[74–75] The modern antimicrobial agents, isoniazid and pyrazinamide, were developed as nicotinamide analogues.^[7,74,76]

Thus, in summary, the human tryptophan–kynurenine–nicotinamide pathway is moderately active during HIV infection and highly active during TB disease, yet it also produces an end-product (nicotinamide), analogues of which are known to inhibit TB symptoms.^[74] These features are difficult to reconcile.

How nicotinamide analogues inhibit the disease is not clear, and may not involve direct antimicrobial suppression or killing, as *M. tuberculosis* utilizes nicotinamide within its own metabolism.^[50] Isoniazid and pyrazinamide induce changes to the NADH/NAD⁺ ratio of the mycobacterium.^[77] Isoniazid and pyrazinamide affect host, as well as mycobacterial, nicotinamide metabolism. Counterintuitively, isoniazid (INH) treatment is a known cause of pellagra, a host niacin (Vitamin B3) deficiency, possibly through competition between INH and niacin.^[74,78–79] The mechanisms of action of nicotinamide analogues such as isoniazid, are multiple and still under investigation after almost a century of use.^[75] Thus, the mechanism by which nicotinamide inhibits TB symptoms remains speculative. Our understanding of nicotinamide metabolism requires a composite picture of host and microbe rather than viewing each organism in isolation. The host and its microbial flora comprise a meta-organism, a “holobiont”.^[80] Our understanding of the nicotinamide pathway of the combined holobiont requires clarification.

During TB latency, it is plausible that the nicotinamide pathway of the holobiont is in equilibrium. Upregulation of IDO during active disease seems counterintuitive, as one would expect increased host-derived nicotinamide to suppress TB growth. Thus, induction of the IDO pathway concurrent with active TB, raises the question of whether the microbe loses its inhibition by nicotinamide. Alternatively, perhaps the host-derived nicotinamide is being utilized elsewhere too quickly, before it can reach the microbe to keep microbial growth in check. Indeed enzymes upregulated by activated immune cells include CD38, which has NAD⁺ glycohydrolase function.^[42–44] Alternatively, therapeutic nicotinamide analogues may suppress TB symptoms through inhibition of host macrophage function, for example through suppression of alternate macrophage activation, rather than by direct inhibition of microbial growth or microbial killing. Pharmacological drug screening may therefore show differing effects using extracellular mycobacterial growth models or growth in macrophages. Such questions focus attention on nicotinamide sharing as a key interaction between the host macrophage and the mycobacterium sharing the same intracellular milieu.

8. Understanding Immunometabolism Requires an Evolutionary Step Further Backwards in Time

The term “intracellular pathogen” is often used to describe bacterial infections within macrophages, such as *Chlamydia pneumoniae*, *Legionella pneumophila*, *Brucella abortus*, *Listeria monocytogenes*, and *M. tuberculosis*. Interestingly, these infections are associated with a particular type of host cellular metabolism, known as oxidative glycolysis or Warburg-like metabolism, within the macrophage.^[38–39] Warburg-like metabolism is characterized by glycolysis in the cytoplasm with downregulation of oxidative phosphorylation in the electron transport chain of the

mitochondrion. Interest in such mitochondrial “tuning” of immune cells has ignited a “renaissance of cellular metabolism”.^[24]

Historically, Warburg and others postulated the switch from oxidative phosphorylation (involving the full Krebs cycle and electron transport chain within the mitochondrion) to aerobic glycolysis as the root cause of malignancy.^[40,81–83] Subsequently, Warburg metabolism in cancer has been considered merely a malignancy-associated phenomenon.^[84] Despite the limitations on the amount of adenine triphosphate (ATP) that can be produced through glycolysis alone (2 ATP molecules produced per glucose molecule compared with 36 ATP molecules via the Krebs cycle and oxidative phosphorylation), rapidly dividing cells undergo glycolysis without completing electron transfer to oxygen, even when oxygen is available to them. Rapid glycolysis leads rather to increased fermentation of glucose to lactate in the cytoplasm.

An evolutionary perspective reminds us that the ancient conserved partnership between a cell and its mitochondria is fundamental to eukaryotic life. Eukaryotes originated from successful endosymbiosis of an alpha-proteobacterium, which became the mitochondrion, within an archeon.^[85–86] Any interference “divorcing” the cell cytoplasm from its symbiotic mitochondrion may cause the cell to act in a more prokaryotic “unicellular” fashion. Such behavior may underpin various aspects of cancer biology, where a cell acts in a “selfish” manner rather than in a co-operative relationship with neighboring cells.

Cells of both the innate and adaptive immune system demonstrate Warburg-like metabolism when activated, including macrophages, dendritic cells and neutrophils, T and B lymphocytes.^[24] As with many rapidly dividing cells, dividing immune cells display Warburg-like metabolism. Importantly, macrophage phenotype is associated with Warburg-like metabolism. Classically activated M1 macrophages show Warburg-like metabolism, while alternatively activated M2 macrophages undergo oxidative phosphorylation in their mitochondria.^[24,39,87]

M1 macrophage activation is associated with expression of the transcription factor Hypoxia inducible factor-1 α (HIF-1 α), which regulates the transcription of glucose transporter 1 (GLUT1), promoting the switch to oxidative glycolysis, and induction of inducible nitric oxide synthase. Alternatively activated M2 macrophages however express HIF-2 α , which induces a different set of genes including arginase.^[24]

Shi and colleagues have shown induction of Warburg-like metabolism during mouse^[88] and human Tuberculosis.^[89] Shi and colleagues have also reviewed biphasic macrophage dynamics during TB infection, where they describe early infection leading to M1 polarization and later dynamics showing polarization to M2 phenotype.^[90] Also supportive of the link between mycobacterial infection and Warburg metabolism is the finding that vaccination with the Bacillus Calmette–Guerin strain of *Mycobacterium bovis*, the globally used anti-Tuberculosis vaccine, reduced hyperglycaemia in type 1 diabetes through induction of aerobic glycolysis.^[91]

Clinical applications of Warburg-like metabolism in TB include use of ¹⁸F-fluorodeoxyglucose (FDG)-Positron emission tomography (PET) scanning.^[92] FDG-PET scanning demonstrates increased uptake of the glucose analogue, FDG. Metabolically active tissues with rapid glucose uptake, such as the brain and

heart, label strongly. FDG-PET has long been used to detect “hot lesions” of tumors. Increased glucose uptake can be a surrogate marker for Warburg-like metabolism^[84,93] or due to anaerobic respiration resulting from hypoxia.^[93] Increased glucose uptake may also be due to stromal cells surrounding the malignancy, rather than due to malignant cells themselves, termed a reverse-Warburg effect.^[84,93] FDG-PET is now finding application to detect active TB granulomas.^[94–95] Lesions during active TB display high glucose uptake via FDG-PET. Findings remain preliminary and some authors have reported lesions with elevated FDG-PET in asymptomatic or cured patients.^[96] The role, if any, of a reverse-Warburg effect in TB granulomas has not been addressed. Thus, the extent of Warburg-like metabolism in TB can only be partially understood through FDG-PET technology.

In summary, it appears that immunometabolism may be a key feature of TB pathogenesis, marking the switch between macrophage states permissive for active versus latent disease. Most evidence associates active disease with a shift away from Warburg-like metabolism (such as increased markers of alternative macrophage activation and IDO expression during human TB). Uncertainties remain, including whether FDG-PET imaging suggests Warburg-like metabolism or reverse-Warburg effect in active TB lesions. Whether the metabolic shift away from Warburg-like metabolism is a response to active *M. tuberculosis* (and other intra-macrophage bacteria) or the cause of disease symptoms awaits clarification. Certainly, the typical TB symptoms of weight loss, fever, and night-sweats point towards mitochondrial energetics as central to TB symptomatology.

9. Mitochondrial Function that Ensures Human Reproductive Fitness may Predispose to Tuberculosis

While oxidative glycolysis has long been recognized as a feature of rapidly dividing cells, an evolutionary perspective begs more thought. Why would a cell “switch off its mitochondria” at a time when it requires energy for division? In order to answer this question, we can perhaps focus less on ATP and become more “hydrogen-focused”, that is, we should look harder at the role of NADH generation via host metabolism. The NAD⁺ centric view has been termed “the NAD world” and follows from the “hydrogen hypothesis” of eukaryotic life, which suggests that eukaryotic life evolved based on symbiosis for hydrogen metabolism.^[97–99]

Mitochondria play a key role in the activation of innate inflammatory pathways and inflammatory cell death.^[100] Nicotinamide and the NADH/NAD⁺ ratio are conserved mediators of host immunity, playing a key role in plants, fungi, and animals.^[101–102] Viral pathogens mediate numerous effects through interactions with host mitochondria.^[103–104] The IDO pathway is the mechanism by which the host generates nicotinamide and therefore may be integral to sensing the NADH needs of the cell.

Cells invest heavily in maintaining fidelity of their mitochondrial DNA. Mitochondrial DNA is passed on via the maternal line only. During sperm–egg fusion in mammalian reproduction, the egg actively degrades any sperm-derived mitochondria, by various processes including mitophagy, a form of allophagy.^[105–106] A good match between nuclear and mitochondrial genes may be required for optimal functioning of enzymes of the electron

transport chain.^[107–109] The mechanisms by which the female mitochondria recognize the foreignness of invading paternal mitochondria remains obscure.^[106] Maintenance of mitophagy as an essential feature of mammalian reproduction may shed light on why certain prokaryotic organisms (resembling foreign mitochondria) may induce inflammatory reactions in the host. NAD metabolism, specifically the NAD⁺/NADH ratio, is emerging as key to understanding multiple diseases including diabetes, obesity, neurodegeneration and cancer.^[41,48,83] An intensified effort to understand mitochondrial factors required for successful reproduction, including how maternal mitochondria determine “foreignness” of sperm counterparts, may illuminate our shadowy understanding of why some organisms are viewed as commensals by our immune systems while others invoke inflammatory responses. Mitochondrial control of cellular metabolism, directing shifts between Warburg-like metabolism and oxidative phosphorylation, may be a relic of mitochondrial actions required for recognition and destruction of foreign mitochondria during sperm–egg fusion.

The IDO-catalyzed nicotinamide synthesis pathway may reflect changes in the NADH/NAD⁺ demands of the mitochondria.^[110] The IDO catalyzed pathway may therefore represent flux between Warburg-like metabolism and oxidative phosphorylation.

Immune tolerance, reproductive fitness, immunometabolism and TB therapeutics converge on IDO-catalyzed nicotinamide production. A clearer image of this pathway, during both latency and reactivation of *M. tuberculosis*, may shed light on why, after centuries of co-evolution, human genes resulting in TB susceptibility have not been subjected to negative selection. Strong reproductive advantage must be linked with the same pathways that lead to TB susceptibility.

10. The IDO Pathway Presents Therapeutic Targets

In addition to the nicotinamide analogues long used as standard antitubercular treatment, IDO inhibition by other pharmacological agents has been explored in pre-clinical and clinical trials, largely for cancer.^[6,111] IDO inhibition is in trials for HIV control^[21] and in animal and in vitro models for Tuberculosis therapy. For example, in a mouse TB model, imatinib, a licensed tyrosine kinase inhibitor, reduced bacterial burden and the number of TB granulomas^[112] with a mode of action thought to be via IDO inhibition.^[113] In a macaque TB model, IDO inhibition reduced mycobacterial burden and pathology.^[114] Other relevant strategies have included using inhibitors targeting mycobacterial tryptophan synthesis^[40,115] or the mycobacterial NAD⁺ synthesis or recycling pathways.^[50,72,77]

Monitoring of endogenous NAD synthesis via the IDO pathway holds promise for TB diagnosis and monitoring effective TB treatment. Deeper attention to the host IDO-catalyzed pathway may present new targets for rational drug design or new trials of already licensed drugs.

11. IDO Reflects Switches in Macrophage Metabolism that Mediate TB Pathogenesis

We have summarized literature showing that elevated IDO activity is regarded as a feature of M2 macrophage activation.^[116] We

and others have shown elevated IDO activity at the onset of active TB. In certain elements of the literature, M1 macrophages have been associated with TB control while M2 macrophages have been thought to be associated with loss of TB control^[117–118] although conflicting reports exist.^[119] Authors have reported a switch from oxidative phosphorylation, associated with the M2 phenotype, to aerobic glycolysis, a feature of the M1 phenotype, during active TB, although this work is largely in non-human models.^[88–90] A conundrum thus remains—how active TB in humans can be associated both with M1 macrophages, undergoing Warburg-like metabolism, as well as M2 macrophages with elevated IDO activity. Directed investigations may be able to illuminate our understanding of whether switching between Warburg-like metabolism and oxidative phosphorylation is causally related to disease onset.

12. Conclusions and Prospects

From an evolutionary medicine perspective, a condition that upregulates IDO activity may hypothetically result in increased fertility of a population, due to the role of IDO in supporting healthy pregnancy. A genetic predisposition towards high IDO activity may overlap with genes for TB susceptibility. Alternatively, infection with latent *M. tuberculosis* may upregulate IDO activity, giving a reproductive advantage to carriers of the organism. These hypotheses may explain why populations susceptible to *M. tuberculosis* infection persist, despite increased mortality from active TB disease.

The human host and its intra-macrophage mycobacterium co-exist in a state of equilibrium during *M. tuberculosis* latency. This equilibrium likely relates to the metabolism of nicotinamide, NAD⁺ and NADH. Shifts in cellular metabolism may play a causal role in development of active Tuberculosis. A large body of animal and human studies show that during active TB, IDO activity is markedly elevated. We hypothesize that flux through the IDO-catalyzed tryptophan degradation pathway may be a surrogate indicator of shifts to and from Warburg-like metabolism.

In summary, the tryptophan–kynurenine–nicotinamide pathway deserves intense scrutiny regarding its potential to reveal novel diagnostics and therapeutic targets for tuberculosis. The pathway is central to immune tolerance as well as fertility, an attractive target from an evolutionary perspective. The pathway is also a key contributor to cellular NAD levels, relevant in a NAD-centric worldview. More emphasis on understanding the holobiont's NADH/NAD⁺ cycle and feedback loops may elucidate unanswered questions.

An evolutionary perspective on mitochondria as ancient symbionts within a eukaryotic cell suggests that mitochondrial sensing of “foreign” mitochondria during sperm–egg fusion may be our compass to direct a deeper understanding of mitochondrial energetics during infectious disease.

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Conflict of Interest

The authors declare no conflict of interest.

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